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Tim J.G. Wilms

PhD Thesis

DTU Aqua
National Institute of Aquatic Resources

Restoration and non-invasive monitoring of geogenic reefs in temperate waters

Section for Coastal Ecology

Tim J.G. Wilms
PhD Thesis

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Cover

Deployment of a remote underwater video system to monitor a reef restoration site in Flensborg Fjord, Denmark. Picture kindly provided with permission by Casper Tybjerg (TTF; www.ttf.dk).

Preface

This thesis is submitted as part of the requirements for the degree of Doctor of Philosophy (PhD) at the Technical University of Denmark. The research presented was carried out at the National Institute of Aquatic Resources (DTU Aqua) within the Section for Ecosystem-based Marine Management (year 1 and 2) and the Section for Coastal Ecology (year 3). The PhD project was conducted under the supervision of Jon C. Svendsen (main supervisor), Josianne G. Støttrup (co-supervisor) and Henrik Baktoft (co-supervisor). In addition, research was carried out in collaboration with the Section for Marine Living Resrouces, DTU Aqua Silkeborg, under the supervision of Prof. Einar E. Nielsen and Dr. Magnus W. Jacobsen.

Hørsholm, Denmark

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Tim J.G. Wilms

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I want to express my gratitude to the assessment committee: Niels Jepsen, Peter Grønkjær and Even Moland – thank you for taking the time to read and evaluate my work.

Many thanks to Bo Mammen Kruse for all the assistance during the field periods in Sønderborg, for letting us use your garden and shed to store the countless anchors, buoys and entangled ropes, and most importantly, for being so actively involved in the ongoing efforts to restore reef habitats in Denmark. Your contribution to this thesis work has been invaluable! A big thanks goes out to the Sønderborg field crew for all the hard work and fun times: to Pauli, Nicole, Jeannet, Charlotte, Oliver, Johan S., Elisenda, Carl, Océane, Florence and Diarmuid – the extended periods spent in the outback of Denmark would definitely not have been the same without you guys! Additional thanks to Mathilde, Gesine, Johan B. and Camilla for your endurance and dedication towards spending long hours in front of the screen counting fish.

Work at the office (at least in pre-COVID times) would not have been the same without my office mates and great colleagues: Sieme, Marie, Ciaran, Kat, Elliot, Gildas, Kristian, Aurore, Daniël, Esther, Tim, Karin, Louise and Neil – thanks for the good times and for all the help and useful insights whenever needed. Special thanks to Christian K. for the long (but fun!) sessions we spent on modelling (both at DTU and in Norway) and for all your help with setting up the HPC environment, the bulk of the analyses conducted in this thesis would probably have taken another decade to complete without your assistance! I have also been fortunate enough to collaborate with the genetics crew from Silkeborg: thanks to Brian and Einar for introducing me to the exciting world of eDNA, and Magnus: a big thanks to you especially for all the time you dedicated to guiding me in the challenging task of interpreting eDNA results! Additional thanks go to Lis, Linda, Marianne, Helle N. and Helle K. for helping me with countless administrative issues – to Chris and Leif for setting up my work space and for helping out with setting up many of our visiting students – and to Karin and Ken for your guidance during the final weeks.

English summary

Coastal marine habitats are among the most productive ecosystems on the planet that fulfil important nursery functions for marine organisms and offer numerous benefits to society such as food production, coastal protection and carbon sequestration. Yet, with an estimated 40% of the global population living within 100 km's of the coast, these habitats also face enormous pressure from anthropogenic factors including pollution, urban development and agricultural runoff. The Baltic Sea provides a pertinent example of how a long history of intense human pressures can result in one of the most degraded coastal systems across the globe. High levels of nutrient runoff from land have turned the Baltic Sea into the largest man-made hypoxic area in the world, while systemic overfishing and habitat degradation have triggered a collapse in commercially-important Baltic cod. Geogenic (i.e. rocky) reefs constitute an important type of coastal habitat in the Baltic Sea, utilized by a wide variety of species for foraging, shelter and reproduction. However, century-long extraction of geogenic reefs to serve as construction material has degraded or completely removed large reef areas in the Baltic Sea with detrimental effects to associated flora and fauna. Whereas degraded biogenic habitats can often be recovered by excluding the pressure from the system (e.g. through protected areas), the removal of geogenic material from the seabed constitutes a permanent shift in habitat availability for reef-associated taxa that warrants active intervention for the habitat and its functions to be restored. Such restoration efforts rely heavily on empirical evidence to identify optimal restoration strategies given limited time and resource availability, which highlights the importance of robust sampling designs and consistent, long-term monitoring studies.

      The aim of this thesis is to advance the relatively young scientific discipline of marine habitat restoration by focusing on two independent restoration projects in the Western Baltic Sea (WBS) and by exploring the use of various non-invasive monitoring techniques to document marine communities inhabiting vulnerable habitats. Restoration efforts can potentially benefit from theories and concepts emerging from fields such as conservation biology and reserve design, yet evidence of synergies with restoration ecology are scarce. Following a general introduction and a synopsis of the main research papers in Chapters 1 and 2, respectively, Chapter 3 investigates if a long-standing debate in conservation biology, the dilemma whether single large or several small (SLOSS) habitats promote biodiversity, has important implications for restoration ecology. Accordingly, boulder reefs were restored in a 'single large' (SL) and 'several small' (SS) design and monitored using remote underwater video systems (RUVS) in a before-after control-impact (BACI) sampling design. Results revealed that both reef designs strongly promoted the abundance of gadoids (mainly cod *Gadus morhua* and saithe *Pollachius virens*), yet small-bodied mesopredators were largely associated with the SS reefs. These findings are discussed in detail regarding the potential of reef restoration to contribute to re-establishing top-down control by predatory fishes and the wider implications of the documented SLOSS effects for restoration efforts in general.

      A widely used extension of RUVS monitoring involves the inclusion of bait (BRUVS) that attracts marine organisms to the camera's field of view to enhance the efficiency of sampling efforts, by reducing potential zero inflation (i.e. samples void of detectable organisms) and increasing the power of statistical

inference. The BRUVS method has raised concerns regarding sampling biases, including a potential risk of obscuring species-habitat associations (SHAs) by attracting organisms away from their naturally preferred habitat. Chapter 4 investigates this concern through a comparative assessment of SHAs for seven common Baltic species as recorded by RUVS and BRUVS. We demonstrate that BRUVS are capable of capturing SHAs previously documented in the literature and even show some superiority relative to RUVS by detecting established associations that were absent when using RUVS, potentially due to limited statistical power obtained from RUVS data. These results confirm that BRUVS constitute a suitable non-invasive method to document SHAs while producing relatively high species counts, and hence we continue to employ this sampling method in Chapters 5 and 6.

Chapter 5 reports on one of the few efforts carried out to date to restore cobble (rocks with diameter between ~ 6 and 25 cm) reef habitats. While the extraction of marine boulders from Danish waters was banned in 2010, the removal of smaller cobble-sized rocks continues in dedicated areas. We show that cobble reef restoration has the potential to ameliorate local declines in biodiversity and that variations in cobble coverage on the seabed drive a large number of significant correlations among species in the study area. These results provide evidence in favor of stepping up conservation efforts of cobble habitats in the Baltic Sea, as the ongoing removal and degradation of this habitat type continue to put associated reef taxa at risk. Finally, motivated by the relatively time-demanding analysis of video data associated with RUVS monitoring, Chapter 6 explores the potential of emerging environmental DNA (eDNA) techniques to monitor marine taxa and their association with coastal habitats. eDNA sampling offers a rapid and low-cost method to sample target species or entire communities with a high degree of sensitivity, as organisms are detected from DNA traces they release into the surrounding environment that can be collected from seawater samples with little effort. We assess the potential to infer SHAs from eDNA data for four common coastal species through a direct comparison with subsequently deployed BRUVS and show that eDNA sampling is capable of detecting SHAs for sedentary species on a fine spatial scale. Given the mosaic seabed characteristics of the study area, our findings suggests that eDNA traces of these sedentary species were highly localized, yet the lack of strong correlations between eDNA signals and fish counts from BRUVS concurrently highlights the ongoing challenge of inferring relative species abundance from eDNA data.

Collectively, results from this thesis work demonstrate that geogenic reef restoration can benefit marine biodiversity and promote the abundance of commercially important species such as cod and other gadoids. The BACI sampling design and Bayesian inference play a central role throughout the thesis and we show how the two components can be effectively combined to disentangle restoration effects from natural system fluctuations while offering an intuitive probabilistic interpretation of results. We argue that eDNA sampling represents a powerful supplement to (B)RUVS monitoring studies by providing high sensitivity for the detection of target species and recommend in-tandem use of these methods to more effectively guide marine conservation and restoration efforts.

Dansk resumé

Kystnære habitater er iblandt de mest produktive økosystemer på jorden, der fungerer som opvækstområder for marine organismer og er fordelagtige for samfundet via fødeproduktion, kystbeskyttelse samt optag af kulstof. Men, med omkring 40% af verdens befolkning bosiddende indenfor 100 km fra kysten, så udsættes de kystnære habitater også for menneskelige presfaktorer heriblandt forurening, udvidelse af byer og udledninger fra landbruget. Østersøen er et relevant eksempel på, at kraftig påvirkning fra menneskelige presfaktorer igennem lang tid kan resultere i et af de mest forringede kystnære systemer i verden. Store udledninger af næringsstoffer fra land har bevirket, at Østersøen er blevet til det største menneskeskabte område med iltsvind i verden, samtidigt med at systematisk overfiskeri og habitatforringelse har betydet, at bestanden af den kommercielt betydningsfulde østersøtorsk er kollapset. Stenrev udgør et vigtigt kystnært habitat i Østersøen, der anvendes af en lang række arter til fouragering, skjul og reproduktion. Imidlertid har stenfiskeri igennem et århundrede til byggeri forringet eller helt fjernet stenrev i store områder af Østersøen med ødelæggende konsekvenser for den associerede flora og fauna. I modsætning til biogene rev, der kan reetableres ved at stoppe systemets presfaktorer (f.eks. via beskyttede områder), så betyder stenfiskeri fra havbunden en permanent ændring i forekomsten af habitater for rev-associerede arter, der forudsætter aktive restaurering, hvis rev-habitaterne og deres funktioner skal genskabes. Den slags restaurering er meget afhængig af empirisk bevismateriale til at identificere optimale restaureringsstrategier, givet begrænset tid og ressourcer, hvilket understreger behovet for design af robust dataindsamling samt studier med konsistent og langvarig monitering.

Formålet med denne afhandling er at styrke den forholdsvist nye videnskabelige disciplin, marin habitatrestaurering, ved at fokusere på to uafhængige restaureringsprojekter i den vestlige Østersø og ved at udforske anvendelsen af forskellige ikke-skadelige moniteringsteknikker til at dokumentere marinbiologiske samfund, der forekommer i sårbare habitater. Restaureringstiltag kan potentielt drage fordel af teorier og koncepter stammende fra discipliner som bevaringsbiologi og design af beskyttede områder, men synergi med disciplinen restaureringsøkologi forekommer sjældent. Efter en generel introduktion og en gennemgang af de vigtigste videnskabelige artikler i henholdsvis kapitel 1 og 2, undersøger kapitel 3 en langvarig debat inden for bevaringsbiologien - nemlig dilemmaet om enkelte store eller mange små (ESEMS) habitater fremmer biodiversiteten mest - kan være vigtigt i forhold til restaureringsøkologi. Med det formål blev stenrev restaureret som enkelte store (ES) stenrev og som mange små (MS) stenrev og moniteret med undervandskameraer (UVK) opsat på havbunden i rev-områderne og kontrol-områderne, der blev undersøgt, både før og efter stenrevene blev anlagt (betegnes BACI-design). Resultaterne viste, at begge rev-designs (ES og MS) kraftigt fremmede forekomsten af torskefisk (hovedsageligt torsk *Gadus morhua* og sej *Pollachius virens*), men små rovdyr som kutlinger o. lign. var hovedsageligt forbundet med MS-stenrevene. Resultaterne diskuteres i detaljer i forhold til, om restaurering af stenrev kan bidrage til at genskabe betydningen af store rovfisk længere nede i fødekæden samt de mere generelle effekter af ESEMS i forhold til restaureringstiltag.

En udvidet anvendelse af UVK-monitering bruger agnede kameraer (AUVK), som tiltrækker marine organismer til kameraets synsfelt for at øge effektiviteten af dataindsamlingen. Tiltrækning af organismerne reducerer antallet af optagelse uden registrerede organismer og styrker derved de statistiske analyser. Men anvendelse af AUVK kan muligvis sløre sammenhænge mellem forekomster af habitater og arter (SHA'er), fordi agnen kan tiltrække arter til habitater, der ikke er arternes foretrukne habitat. Kapitel 4 undersøger denne mulighed nærmere ved at sammenligne SHA'er for syv almindelige arter i Østersøen, der blev registreret af UVK og AUVK. Sammenligningerne viste, at AUVK kan bruges til at registrere SHA'er, der er dokumenteret tidligere, og at AUVK endda kan være et bedre værktøj sammenlignet med UVK, fordi AUVK registrerede kendte SHA'er, der var fraværende ved anvendelse af UVK, potentielt fordi styrken af den statistiske analyse af UVK data var begrænset. Resultaterne bekræfter at AUVK udgør en velegnet ikke-skadelig moniteringsteknik til at dokumentere SHA'er med relativt højt antal registrerede arter. Derfor blev AUVK ligeledes anvendt til dataindsamling i kapitel 5 og 6.

Kapitel 5 afrapporterer en relativt sjælden undersøgelse af restaurerede rev-habitater bestående af ral-sten med en diameter på omkring 6 – 25 cm. Stenfiskeri efter kampesten blev forbudt i Danmark i 2010, men indvinding af ral-sten fortsætter i dedikerede områder. Undersøgelsen viste, at restaurering af ral-rev har potentiale til at afhjælpe lokale reduktioner i biodiversiteten, og at varierende dækningsgrader af ral-sten på havbunden er korreleret med forskellige arter i studie-området. Resultaterne understøtter muligheden for at øge beskyttelsen af ral-habitater i Østersøen, fordi den nuværende fjernelse og forringelse af ral-habitater fortsat truer de associerede arter.

Kapitel 6 er motiveret af de tidskrævende video-analyser af undervandsoptagelserne. Kapitlet undersøger potentialet ved en nyere metode, der betegnes eDNA, til monitering af marine arter og deres tilknytning til kystnære habitater. Dataindsamling forbundet med eDNA er baseret på relativt billige og hurtige metoder til at dokumentere specifikke arter eller hele artssamfund med høj sandsynlighed for registrering, fordi organismerne registreres via DNA-spor, der frigives til det omkringliggende miljø. DNA-sporene indsamles nemt via vandprøver, der efterfølgende analyseres. Undersøgelsen omfattede fire almindelige kystnære arter, hvor eDNA blev forsøgt anvendt til at udlede SHA'er, og sammenlignet med tilsvarende SHA'er udledt via parallelt anvendte AUVK. Resultaterne viste, at eDNA kan bruges til at registrere SHA'er hos mobilitets-begrænsede arter med god rummelig opløsning. Baseret på studie-områdets mosaik af havbunds-typer antyder undersøgelsen, at eDNA-spor af de mobilitets-begrænsede arter var meget lokale - men der var ikke stærke sammenhænge mellem eDNA-registreringerne og antallet af fisk, der blev registreret med de anvendte AUVK'er. Resultatet fremhæver udfordringerne ved at udlede relative artsforekomster via eDNA data.

Samlet set så viser arbejdet med afhandlingen, at stenrev kan restaureres succesfuldt til gavn for biodiversitet og kommercielt set vigtige arter som torsk og andre torskefisk. BACI design og Bayesian statistiske metoder er vigtige elementer i afhandlingen, der viser, at de to værktøjer kan kombineres effektivt til at adskille restaureringseffekter fra naturlige fluktuationer i systemet og angive

sandsynligheder for intuitive fortolkninger af resultaterne. Arbejdet fremhæver endvidere, at eDNA udgør et godt supplement til (A)UVK moniteringsstudier, fordi metoden muliggør registrering af specifikke arter med høj sandsynlighed for detektion, hvilket understøtter kombineret anvendelse af begge metoder til effektive tiltag forbundet med beskyttelse og restaurering af marine områder.

Contents

Chapter 1

General introduction

1.1. Glaciation of the Baltic basin: A brief history

The seabed of the Baltic Sea has a relatively young yet rich and dynamic geological history that has fascinated Quaternary geologists in Baltic countries for generations (Björck, 1995). The geodiversity of the Baltic seabed has a glacial origin and resulted from a variety of glacial and post-glacial processes that have gradually shaped the geomorphic features of the Baltic basin since the onset of the Pleistocene (*ca.* 2.6 million years BP; Mangerud, Jansen, & Landvik, 1996; Kaskela & Kotilainen, 2017). Out of several major glaciation periods that have occurred during the Pleistocene, the Last Ice Age (i.e. the Weichselian glaciation) and, in particular, the Last Glacial Maximum (LGM; *ca.* 21,000 years BP) have been most extensively studied (Mangerud et al., 1996; Lasberg, 2014). During the LGM, the Scandinavian ice sheet (also known as the Fennoscandian ice sheet) constituted the largest ice sheet in Eurasia and covered extensive parts of the Northern hemisphere, including the entire Baltic Sea basin (Hughes et al., 2016; Stroeven et al., 2016). The gradual advance of the ice sheet induced erosion of underlying sediment and subsequent accumulation and transportation (Fig. 1.1) of unsorted glacial debris (known as glacial till) of Scandinavian origin into the Baltic basin. The thick and extensive ice coverage forced immense pressure on the underlying surface and, depending on ice thickness, caused depressions of the lithosphere of up to hundreds of meters below the ice (Andrén et al., 2011; Steffen, Kaufmann, & Lampe, 2014). Recurring glacial advances and recessions therefore had a huge impact on the entire basin, and complex interactions between isostatic rebound (i.e. uplift of ice-free land), deglaciation processes and major changes in sea-level gave rise to a series of lacustrine and brackish precursors of the present Baltic Sea (e.g. Baltic Ice Lake, Yoldia Sea and Ancylus Lake; described in detail in Björck, 1995; Andrén et al., 2011).

From the LGM onward, the Scandinavian ice sheet receded northward and by approximately 10,000 years BP the entire Baltic basin was deglaciated (Andrén et al., 2011). During the deglaciation, glacial till deposits became exposed in the basin at ground and terminal moraines and were actively reworked by wind forces. The subsequent filling of the basin during the marine transgression phase was rapid until about 7,000 years BP (Diesing & Schwarzer, 2006), during which currents and waves further shaped and redistributed the glacial deposits. As a result, large-sized rocks (i.e. cobbles and boulders following the classification of grain size classes by Wentworth, 1922; Table 1.1) were deposited close to

original source (also termed lag deposits), while finer sediment was transported further away in neighboring areas (Diesing & Schwarzer, 2006; Papenmeier et al., 2020). Notably, as the Scandinavian ice sheet extended beyond the southwestern Baltic coastlines during the LGM (Svendsen et al., 2004), this enabled the formation of lag deposits along and in close proximity to the shore during subsequent deglaciation (Diesing & Schwarzer, 2006). In contrast, the Scandinavian ice sheet did not extend as far as the present Danish and German parts of the North Sea, implying that lag deposits there are of Saalian origin or older (> 130,000 years old), and were therefore modified significantly longer by wind, waves and tidal forces (Diesing & Schwarzer, 2006; Michaelis et al., 2019). This difference in glaciation history between the two basins has resulted in an important contrast between the distribution of hard substrates within the North Sea and Baltic Sea today. While cobble and boulder habitats are scattered at low densities in the North Sea and mainly restricted to offshore areas of depths between 20-45 m (e.g. Borkum Reef Grounds; Coolen et al., 2015; Michaelis et al., 2019; Papenmeier et al., 2020), these
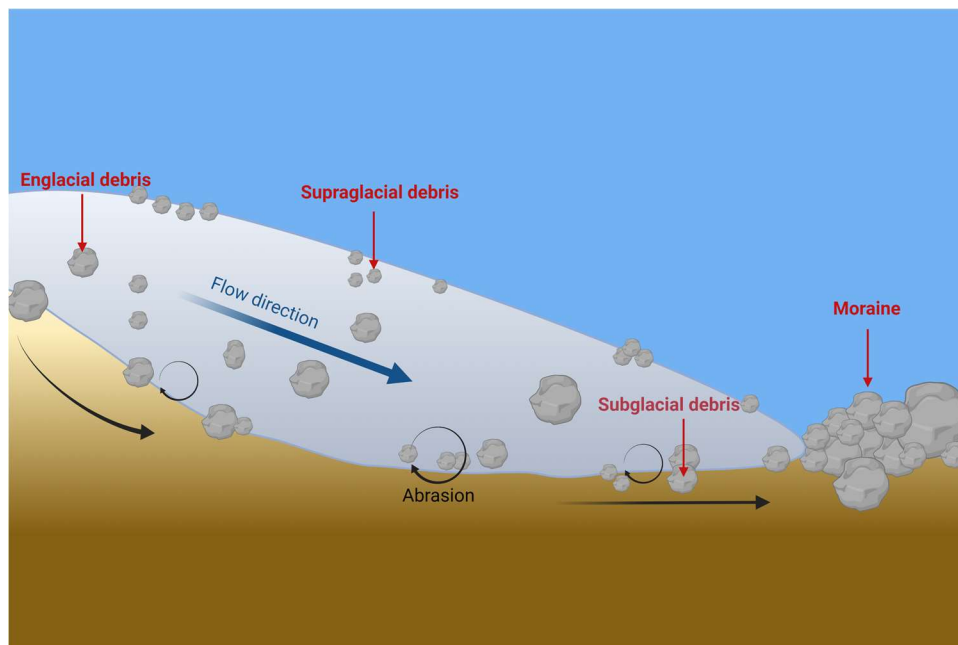
Figure 1.1 – Conceptual drawing of erosion, transportation and deposition of glacial debris within the ice and at the terminus during the advance (and subsequent retreat) of an ice sheet. Glacial debris is transported on top of the ice (supraglacial), within the ice (englacial) and under the ice at the base (subglacial). Rocks embedded at the base grind away the bedrock underneath (i.e. abrasion), thereby acting as sandpaper and altering the landscape as the glacier advances. Upon retreat of the glacier, the unsorted debris is deposited and forms different types of moraines, e.g. evenly distributed supraglacial moraines or large accumulations of rock at the terminal moraine. Created with BioRender (www.biorender.com).

habitats are found at relatively higher density in shallow coastal areas (between 5-15 m) in the Baltic Sea (Diesing & Schwarzer, 2006; Papenmeier et al., 2020). Throughout the present day Baltic basin, many of these lag deposits remain largely covered by limnic sediments originating from the Baltic Ice Lake (~ 12,600 – 10,300 years BP) and organic-rich mud deposited throughout the final Baltic Sea phase (Andrén et al., 2011; Papenmeier et al., 2020). Yet, seafloor abrasion induced by hydrodynamic forces continues to expose "new" cobble and boulder habitats today (Bohling et al., 2009; Schwarzer et al., 2014; von Rönn et al., 2021).

Collectively, the various brackish and lacustrine stages of the Baltic basin, characterized by large fluctuations in salinity and sea levels, highlight a very dynamic history within an extremely short geological timeframe. While large-scale environmental gradients shaped by post-glacial processes have relatively stabilized during the last 3,000 years (Snoeijs-Leijonmalm, Schubert, & Radziejewska, 2017), unprecedented changes within the contemporary Baltic Sea system are now occurring on much shorter time scales from centuries to decades.

Table 1.1 – Grain size classes used throughout this thesis after Wentworth (1922) and modified from (Snoeijs-Leijonmalm et al., 2017).

Substrate type	Size class	Lower grain size limit (mm)	
Gravel	Boulder	256	
	Cobble	64	
	Pebble	4	
	Granule	2	
Sand	Very coarse sand	1	
	Coarse sand	0.5	(1/2)
	Medium sand	0.25	(1/4)
	Fine sand	0.125	(1/8)
	Very fine sand	0.0625	(1/16)
Silt	Coarse silt	0.0313	(1/32)
	Medium silt	0.0156	(1/64)
	Fine silt	0.0078	(1/128)
	Very fine silt	0.0039	(1/256)

1.2. The Baltic Sea in present time

1.2.1. Environmental conditions

Today, the Baltic Sea constitutes one of the largest brackish water bodies on the planet with a surface area of 415,000 km^2, equivalent to 0.1% of the total world's oceans in area (Ducrotoy & Elliott, 2008). The Baltic Sea is characterized by a strong horizontal (east-west and north-south) salinity gradient (Fig. 1.2), with salinity levels approaching full marine conditions at the entrance (~ 25 ‰) in the Kattegat all the way down to freshwater conditions (< 1 ‰) in the Gulf of Bothnia and Gulf of Finland (Bonsdorff, 2006). With an average water depth of 57 m, the Baltic Sea is generally very shallow although several deep basins (at most 459 m deep in the Landsort deep) are present in the northeastern parts (Snoeijs-Leijonmalm et al., 2017). The Baltic Sea has a positive overall freshwater budget resulting from large volumes of freshwater runoff from over 200 rivers (Snoeijs-Leijonmalm et al., 2017), inducing a continuous outflow of water in the surface layers (Lehmann et al., 2021). To compensate, episodic inflow of saline waters in the lower layer takes place in the narrow straits connecting the Baltic Sea to the North Sea, which is strongly driven by precipitation and wind conditions (Reissmann et al., 2009; Lehmann et al., 2021). In addition to the characteristic horizontal salinity gradient, these dynamics create a vertical gradient with a permanent
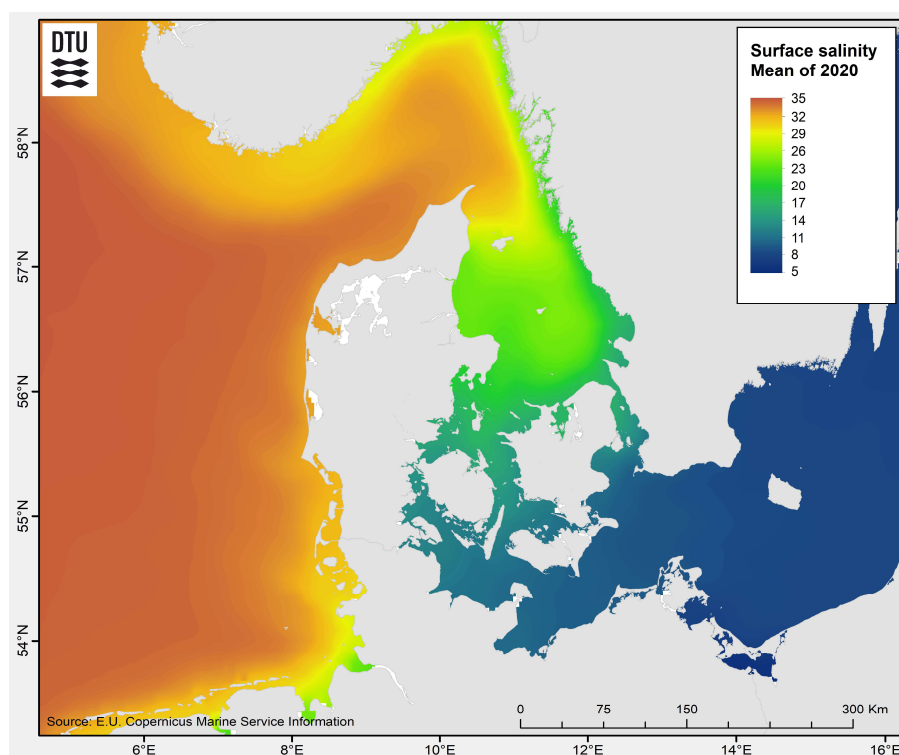
Figure 1.2 – Average sea surface salinity levels within Danish waters and surroundings for 2020, showing a strong east-west salinity gradient with brackish conditions in the SW Baltic Proper towards approaching marine conditions in the Skagerrak and North Sea. Map credits: Jeppe Olsen.

halocline persisting at about 60 m depth in the central Baltic Sea. The halocline restricts vertical exchange between surface layers and bottom waters of low and high salinity, respectively, and deeper waters are only replenished on decadal time scales during Major Baltic Inflows (MBIs), which nowadays only take place about once a decade (Mohrholz et al., 2015). Stagnation of bottom waters results in naturally occurring hypoxia events during which oxygen demand from e.g. benthic fauna or uptake by sediments outpaces the supply of oxygen (Carstensen et al., 2014; Carstensen & Conley, 2019). As a result, periods of deep-water oxygen deficiency occur seasonally in the Kattegat, intermittent in the Gulf of Finland and near-permanent in the central Baltic Sea (Elmgren, Blenckner, & Andersson, 2015). The relatively shallow water depths of the northeastern Gulf of Bothnia prevent the formation of a halocline and this region therefore does not experience the naturally occurring episodes of oxygen deficiency observed elsewhere in the Baltic Sea (Snoeijs-Leijonmalm et al., 2017). Combined, the relatively young age of the present-day Baltic Sea and the extreme environmental gradients imply that few marine taxa have fully adapted to the current salinity regime and the majority of taxa occur at the edge of their physiological limits (Mackenzie et al., 2007; Snoeijs-Leijonmalm et al., 2017).

1.2.2. The importance of hard substrate for Baltic Sea biota
As a consequence of the strong salinity gradient across the Baltic Sea, inhabitant marine taxa decrease in numbers from west to east and are gradually replaced by species of limnic origin (Ojaveer et al., 2010). For fish and benthic invertebrates, the number of species (i.e. species richness) is positively related to salinity (Bonsdorff, 2006; Ojaveer et al., 2010), although some sub-regions such as the Gulf of Finland may actually host a higher species richness compared with more saline areas by providing suitable conditions for both marine and freshwater species (Ojaveer et al., 2010). The seabed of the Baltic Sea is generally dominated by sandy mud and sand in southwestern areas and to a lesser degree by mixed substrate (i.e. consisting of glacial till), which gradually becomes more common in the northeastern parts alongside crystalline bedrock and boulders (Kaskela & Kotilainen, 2017). The glacial till ranges from clay to large rocks (i.e. cobbles and boulders) and constitutes the only source of natural hard substrate in the southwestern parts of the Baltic Sea (Kaskela & Kotilainen, 2017; Franz et al., 2021), surrounded by vast plains of low relief and complexity. As such, these relict deposits can locally create "island-like" stony reef habitats (hereafter "geogenic reefs") that constitute an oasis among a sea of mud to the benefit of reef-associated fish and invertebrate communities (Dahl, Lundsteen, & Helmig, 2003; Beisiegel et al., 2019). The relative positioning of individual cobbles and boulders within the reef system can locally create complex reef structures such as crevices, ledges and cavernous structures that offer shelter from predation for a range of reef taxa. Since highly structured habitats reduce the foraging efficiency of predators (L. Persson & Eklov, 1995; Beukers & Jones, 1998), the survival of juvenile reef taxa is often found to increase substantially with habitat complexity (Lima & Dill, 1990; Scharf, Manderson, & Fabrizio, 2006; Pirtle, Eckert, & Stoner, 2012). Geogenic reef systems therefore constitute important spawning and nursery areas for many temperate fish species (Torn et al., 2017). In addition, the presence of hard

substrate provides a foundation for anchorage of sessile biota that would otherwise be unavailable across the vast areas of soft bottom sediments in the southwestern Baltic Sea. For example, most perennial macroalgal communities (Fig. 1.3) and reef-forming species (e.g. mussels) require hard substrate as a foundation for anchorage, and their development can contribute significantly to regulatory mechanisms such as carbon storage in coastal marine sediments or water filtration (Newell, 2004; Krause-Jensen & Duarte, 2016; Liversage, 2020). The development of biogenic communities further enhances the availability of food and shelter within geogenic reef systems and, combined, these provisional services can attract mobile invertebrates (Pirtle et al., 2012; Mercaldo-Allen et al., 2021), fish (Støttrup et al., 2014; Kristensen et al., 2017; Beisiegel et al., 2019) and marine mammals (Mikkelsen et al., 2013).

Figure 1.3 – Geogenic reefs provide hard substrate for the anchorage of macroalgae (left) and thereby facilitate the development of biogenic communities that enhance the structural complexity of the reef system (right). Photo credits: Casper Tybjerg (left) and Martin Kielland (right).

One example of a marine fish that depends on hard substrate for substantial parts of its lifecycle is Baltic cod (*Gadus morhua;* Fig. 1.4), an iconic predatory fish of high ecological and economic importance in the Baltic Sea region and elsewhere throughout its native range. The eggs of Baltic cod are neutrally buoyant at salinity levels of 14.5 ± 1.2 ‰ (although with significant variations among batches; Nissling, Kryvi, & Vallin, 1994), which is crucial to avoid stressful low oxygen conditions of bottom waters in which eggs are likely to die off (Nissling & Vallin, 1996; Petereit et al., 2014). This implies that neutral buoyancy of Baltic cod eggs can only be achieved below the halocline at depths of 55 m and greater (Wieland, Waller, & Schnack, 1994). Successful spawning of Baltic cod is therefore restricted to the deep Bornholm, Gdansk and Gotland basins, although the latter two basins only periodically attain suitable salinity levels following major inflows from the North Sea (Nissling et al., 1994; Nissling & Vallin, 1996). After hatching, the negatively buoyant larvae undergo a vertical migration through active swimming (i.e. a 'first-feeding migration'), to avoid mortality following exposure to low oxygen conditions in deeper layers and to reach surface waters of higher light intensities and improved feeding opportunities (Grønkjær & Wieland, 1997). Young-of-year (YOY) cod subsequently settle in a diverse range of habitats during the transition phase to the demersal stage (for Baltic cod taking place after reaching a body length of

approximately 50 mm; Hüssy, St. John, & Böttcher, 1997), including sand, gravel and cobble bottoms as well as eelgrass meadows and macroalgal stands (Tupper & Boutilier, 1995b; Cote et al., 2004; Renkawitz et al., 2011; Lilley & Unsworth, 2014). However, post-settlement growth and survival rates strongly depend on habitat type, with growth typically being highest in eelgrass meadows and survival being optimized in coastal reef habitats due to reduced predator efficiency (Tupper & Boutilier, 1995a). Lab experiments have also indicated a preference in juvenile cod for structured (i.e. cobble or vegetated) habitat in the presence of foraging predators and a subsequent reduction in predation risk, possibly due to the obstruction of visual cues (Gotceitas & Brown, 1993; Gotceitas, Fraser, & Brown, 1995; Lindholm, Auster, & Kaufman, 1999). Substrate selection of juvenile cod further appears to be age-specific, with older juveniles showing a stronger association with structured habitats that offer shelter (e.g. high relief boulder habitat) as they lose their mottled body coloration that facilitates a more cryptic lifestyle in areas of low complexity at a younger age (Gregory & Anderson, 1997). Juvenile cod residing in reef habitats have been shown to save energy relative to juveniles occupying bare sand patches, implying that the presence of reef structures may allow juveniles to invest more energy in somatic and gonadal growth to the potential benefit of fitness and reproductive success (Schwartzbach, Behrens, & Svendsen, 2020). As individuals mature further, the optimal ambient temperature for cod decreases and strong diel and seasonal variabilities are observed in the depth and habitat use of adult cod (Seitz et al., 2014; Freitas et al., 2015; Funk et al., 2020). Spawning of Baltic cod takes place during winter in deep waters mainly characterized by muddy bottom and limited availability of hard substrates and prey items, yet these waters provide optimal salinity levels for egg fertilization and buoyancy (Nissling & Westin, 1997). To make up for the low food intake during the spawning period, adult cod exploit the low water temperatures during autumn and spring to stock up and replenish energy reserves, respectively, by foraging in shallow coastal habitats rich in food sources (i.e. eelgrass meadows and vegetated reef structures; Persson et al., 2012; Funk et al., 2020). Finally, rapidly increasing water temperatures and stratification during the summer months once again restrict adult cod to deeper waters of lower temperature, although simultaneously limited by the formation of hypoxic areas in deeper areas during summer, presenting cod with a trade-off between food availability and suitable ambient conditions that may result in low food intake (Freitas et al., 2016; Funk et al., 2020). Collectively, the life cycle of Baltic cod exemplifies the importance of structurally diverse and complex coastal habitats for the growth, survival and successful reproduction of this commercially important species, yet it also underlines the vulnerability of cod populations to the degradation of such habitats resulting from anthropogenic activities.
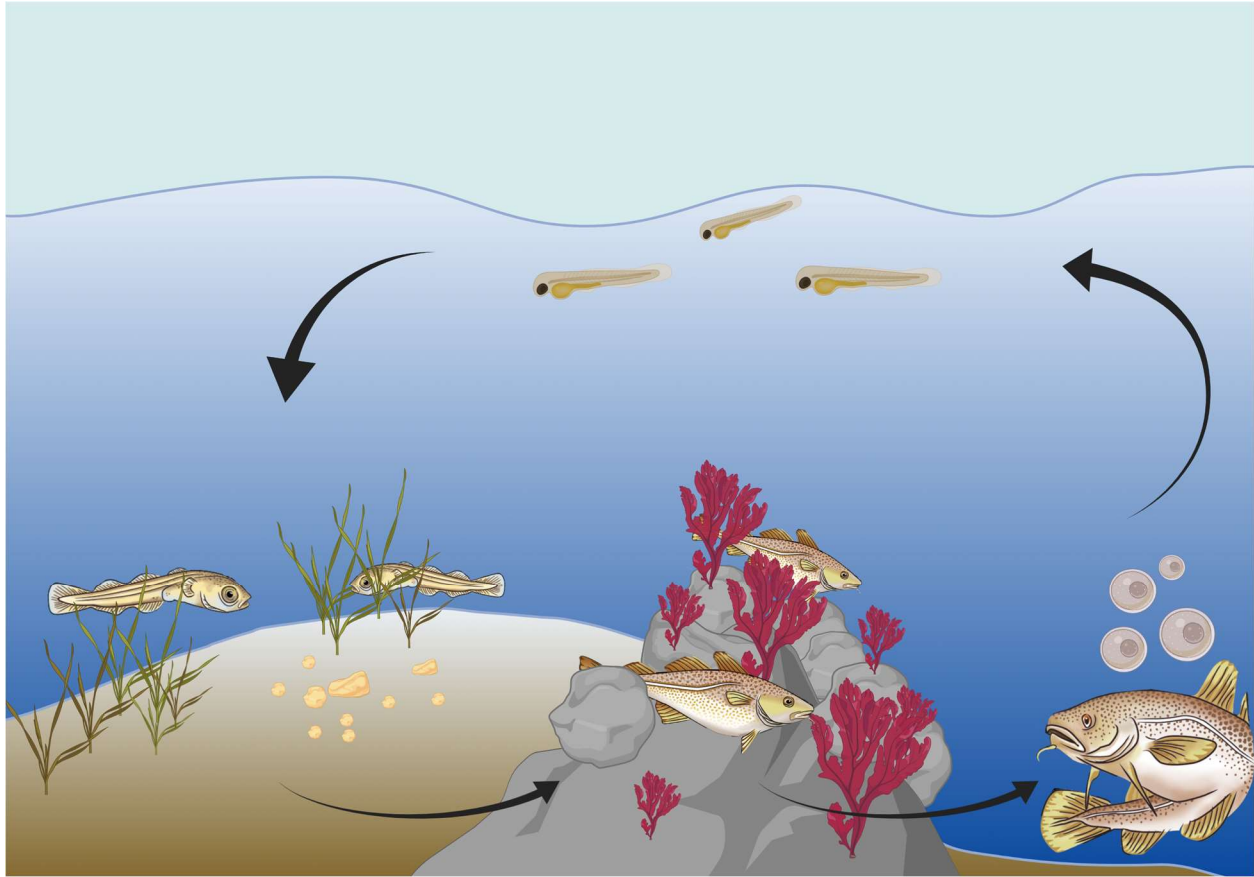
Figure 1.4 – Conceptual drawing showing habitat use by Baltic cod (*Gadus morhua*) throughout its lifecycle. Modified from piscoweb.org/gallery/atlantic-cod-life-cycle. Created with BioRender (www.biorender.com).

1.2.3. The impact of human activities

Early signs of human activities affecting the Baltic Sea date back as far as AD 200, as evidenced by a change in lead composition within sediments from the Eastern Gotland basin reflecting a shift in Roman lead mining activities (Bindler et al., 2009; Andrén et al., 2011). Today, the Baltic Sea faces a unique multi-stressor situation that could serve as an example in terms of impacts and mitigation strategies for other regions potentially facing similar threats in the future (Reusch et al., 2018). One of the main components of this multi-stressor disturbance is hypoxia, i.e. oxygen consumption exceeding the natural supply by the system. As discussed previously, prolonged periods of deep-water hypoxia have occurred naturally in the Baltic Sea over the past millennia due to various atmospheric and hydrodynamic conditions (Zillén et al., 2008). However, it is now evident that the extent of hypoxia in the Baltic Sea has increased 10-fold due to nutrient inputs from land and atmosphere, making the Baltic Sea the largest man-made hypoxic area in the world (Carstensen et al., 2014). The problem is further exacerbated by climate change, as increasing water temperatures increase the oxygen demand of the system through higher respiration rates and simultaneously lead to reduced oxygen solubility in seawater (Rabalais et al., 2009; Carstensen et al., 2014). Fish and crustaceans are most sensitive to hypoxia (Vaquer-Sunyer &

Duarte, 2008) and episodic coastal upwelling can trap large fish schools in hypoxic water and cause mass mortality events visible from shore (Carstensen & Conley, 2019). Hypoxia also strongly influences the diversity of benthic communities and the recent expansion of oxygen-depleted areas has resulted in the loss of benthic macrofauna and severely disrupted benthic food webs (Conley et al., 2009). Another important anthropogenic pressure includes the historic overexploitation of commercially important predatory fish species in the Baltic Sea. For example, the late 1970s were characterized by favorable conditions of Baltic waters for cod spawning and low predation rates by seals, resulting in large numbers of cod larvae and a 'boom' in cod landings (Eero et al., 2011; Orio et al., 2021). However, the subsequent period of overexploitation and declining oxygen and salinity levels caused a decline in cod stocks to the lowest level on record (Köster et al., 2005; Orio et al., 2021). Management actions have thus far resulted in a partial recovery of Baltic cod stocks in terms of abundance (Elmgren et al., 2015), yet a combination of limited invertebrate prey items due to expanding hypoxic areas together with selective fishing on large size classes, has now resulted in slow-growing individuals with deteriorating body conditions (Svedäng & Hornborg, 2014; Eero et al., 2020). The poor status of this top-level piscivore implies a release from predation for small-bodied mesopredators in many areas, which can trigger trophic cascades and cause regime shifts (Eriksson et al., 2011; Burt et al., 2018). Examples include the potential degradation of mussel reef habitat through intensified predation by mesopredators (Christie et al., 2020) and ephemeral algae blooms caused by predation on mesograzers that may result in the loss of eelgrass and perennial algae communities (Moksnes et al., 2008; Östman et al., 2016). While these examples of anthropogenic stressors (although not exhaustive) continue to impact Baltic coastal ecosystems and are therefore extensively documented, direct extractions of geogenic material (i.e. excavations) have also taken place in Danish and German coastal waters for over a century, which have received less attention.

Approximately around 1880, an industry started to develop in Denmark to exploit hard substrates within Danish coastal waters for construction purposes (Dahl et al., 2003; Stenberg & Kristensen, 2015), following similar developments in Germany as early as 1800 (Karez & Schories, 2005). These commercial exploitations were effectively termed the stone fishing industry ("stenfiskeri" in Danish; "Steinfischerei in German) and developed out of a high demand for raw building material and the scarcity of such materials in NW Europe at the time. The material was predominantly used for the construction of piers, jetties and various types of shore protection (Dahl et al., 2003; Kristensen et al., 2017). Anecdotal evidence from stone fishermen has suggested that extractive activities mainly occurred in shallow coastal waters, where marine rocks were most easily accessible (Kristensen, 2016). While rocks were mainly collected by hand from very shallow water during the late 19th century, technological advanced in the mid-1920s facilitated the use of more sophisticated techniques (e.g. by cranes and divers) and thereby the extraction of material from deeper areas (Dahl et al., 2003; Stenberg & Kristensen, 2015). Historical records of the extractive activities in Denmark are mainly limited to old notebooks that were maintained for tax purposes and of which only few have come into the possession of the Danish Nature Agency (Skov- og

Naturstyrelsen; Dahl et al., 2003). Collectively, a precautionary estimate of the area of marine rocks removed by the Danish stone fishing industry between 1950-2000 stands at 40 km^2 (Dahl et al., 2003). Similarly, estimates of extraction from Germany are highly uncertain but suggest that roughly 2.5 million boulders were removed along the coast of Schleswig-Holstein alone (Karez & Schories, 2005) and that a total area of 100 km^2 was removed in the entire Baltic Sea (Zander, 1991; Schwarzer et al., 2014). Since boulder reef habitats have a patchy distribution along shallow coastal waters of the Baltic Sea, these estimates imply substantial degradation of this important habitat type (Franz et al., 2021), as well as for biogenic structures associated with the reefs (Carr, 1994; Støttrup et al., 2014). In addition, while many of the reef areas were not completely removed, extraction of large rocks can impact the stability of reef slopes and significantly alter important reef structures within the remaining reef area. Large rocks can act as a protective shield against abrasion induced by wave and current energy (Healy & Wefer, 1980), and these forces can also transport reef material from unstable slopes into deeper waters and cause a loss of attached macroalgae and associated communities (Stenberg et al., 2013). The loss of cavernous structures formed by larger rocks also significantly decreases the structural complexity of the remaining reef system (Dahl et al., 2003). Indeed, investigations by the Danish National Monitoring Program indicate that only 5 hectares of shallow boulder reefs were left completely untouched (Dahl et al., 2009), suggesting that the stone fishing industry has had substantial impact on coastal reef ecosystems in the Danish and German parts of the Baltic Sea. The stone fishing industry was banned in Germany in 1974 (Bock et al., 2003), yet extractive activities in Danish waters were not prohibited until as recently as 2010 (Kristensen et al., 2017). Various European legislations are currently in place to ensure the conservation of marine habitats in European waters with the goal of reaching a good ecological status (Silva, 2006). This includes the Habitat Directive (HD) that forms the foundation of the Natura 2000 network of protected habitats in which reefs are listed as important habitats (type 1170; The Danish Nature Agency, 2000). To this end, EU member states are required to maintain or restore the protected habitats and their associated species implying that, for coastal reefs permanently removed or altered by human activities, this may be achieved by restoring the reefs and their functions (Støttrup et al., 2017).

1.3. Ecological restoration

1.3.1. Definition and motivations

Ecological restoration is formally defined as "the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed" (Clewell, Aronson, & Winterhalder, 2004). From this definition, a fundamental distinction arises between ecological restoration and other forms of ecosystem repair, namely that the former aims to assist the recovery of natural systems rather than impose a new direction of development (McDonald, Jonson, & Dixon, 2016). This definition, however, does not include the general motivation behind restoration efforts and it has therefore been argued that the definition needs updating to emphasize the duality of restoration (i.e. covering both social and scientific aspects) and thereby more accurately encapsulate all underlying rationales for ecological restoration in the 21st century (Martin, 2017). Ecological restoration encompasses a wide range of activities, including e.g. the replanting of native forests (Smale, Whaley, & Smale, 2001), dam removal to restore flow regimes and natural riverine conditions (Bednarek, 2001), or the addition of boulders and gravel beds in streams to recover spawning habitat for brown trout (Palm et al., 2007). While these types of "active restoration" can be particularly successful at recovering ecosystems or target species, in some cases a "passive restoration" approach without further human intervention, e.g. removal of grazing cattle (Batchelor et al., 2015), may provide an effective and low-cost alternative. The ultimate goal of returning an ecosystem to its historical trajectory over time demands sustained commitment by the parties involved (e.g. ecologists, practitioners and stakeholders), together with a synthesis of expertise to identify the most effective use of space and resources. For example, the most successful method to restore native plant coverage is often not equivalent to the method that restores the largest area given the allocated funding (Kimball et al., 2015). At the same time, restoration efforts should ultimately result in self-sustaining ecosystems that are resistant to external perturbations and require little to no maintenance (Palmer et al., 2005), highlighting ways in which in-depth knowledge of the system may benefit restoration practices. The scientific field of restoration ecology plays an important role in this process, by providing clear concepts, methodologies, tools and models for practitioners to implement (Clewell et al., 2004). Conversely, restoration ecologists often make use of restoration sites as experimental areas to test and further advance ecological theory and thereby provide important information for future efforts (Jordan III, Gilpin, & Aber, 1990; Palmer, Ambrose, & Poff, 1997). While such experiments have facilitated the development of many well-established restoration treatments for terrestrial systems (Kimball et al., 2015), this does not hold true for the restoration within the marine environment.

1.3.2. Marine ecosystem restoration: a young scientific discipline

The ongoing degradation and loss of marine habitats around the globe due to cumulative anthropogenic impacts (Rabalais et al., 2009; Halpern et al., 2019) provides a strong rationale for active restoration of marine ecosystems. However, the field of marine ecosystem restoration is still in its infancy and available

knowledge on factors enhancing or limiting restoration success is therefore relatively scarce for the marine environment (Bekkby et al., 2020). Substantially fewer studies have been carried out on restoring marine habitats relative to terrestrial and freshwater counterparts (Hale et al., 2019), which can at least partly be attributed to the inherent challenges related to working in the marine environment. Restoring and monitoring of marine habitats often involves working in a highly dynamic environment, inducing economic and logistical constraints, and may involve focal species with narrow environmental tolerances for which we have limited in-depth scientific knowledge available relative to e.g. terrestrial species (Bayraktarov et al., 2015; Danovaro et al., 2021). Collectively, these difficulties often result in high variations in restoration success rates within the marine environment (Bayraktarov et al., 2015), which in turn may lower incentives for investments from stakeholders into marine restoration projects (Blignaut et al., 2013). Despite these challenges, the active restoration of marine habitats represents a major part of the interventions needed to rebuild marine life in the twenty-first century (Duarte et al., 2020). Global interest in marine restoration efforts is increasing (Saunders et al., 2020), which is likely to foster ongoing developments of methodologies to restore marine habitats and facilitate empirical studies to test the efficacy of such restoration strategies.

Habitat restoration within the marine environment has thus far mainly involved marine vegetation (Hale et al., 2019), such as seagrass meadows and kelp forests (Layton et al., 2020; Orth et al., 2020). Although there are several examples of successful restoration of seagrass meadows (Leschen, Ford, & Evans, 2010; Orth et al., 2020), global success rates are low (estimated at 37%; van Katwijk et al., 2016) due to a wide range of biotic and environmental challenges. In the Baltic Sea region, high losses of eelgrass (*Zostera marina* L.) seeds have been hampering large-scale restoration efforts of eelgrass meadows and improved restoration techniques are actively being developed to prevent seed loss (Infantes, Crouzy, & Moksnes, 2016; Infantes & Moksnes, 2018). Field experiments have thus far suggested that predation from shore crab (*Carcinus maenas*), physical disturbance from macroalgal drift and bioturbation by lugworms (*Arenicola marina*) are the main causes of seed loss (Valdemarsen et al., 2010; Valdemarsen et al., 2011; Infantes et al., 2016), which could potentially be remedied by seed burial techniques (Marion & Orth, 2012; Infantes et al., 2016). This highlights the importance of consistent post-restoration monitoring to evaluate current methods and of reporting unsuccessful efforts in particular, to develop effective restoration strategies based on past failures. The transfer of knowledge across ecosystems may further improve success rates of eelgrass restoration in the near future, e.g. the use of propagules to enable the restoration of larger areas as is commonly done during mangrove restoration (Vanderklift et al., 2020) or including positive species interactions and feedbacks to facilitate eelgrass growth (Valdez et al., 2020).

Efforts to restore reef ecosystems around the globe are also becoming increasingly popular, yet remain mostly centered around biogenic reefs e.g. transplanting of coral fragments (Fox et al., 2019; Williams et al., 2019) or restoration of bivalve reefs (zu Ermgassen et al., 2020). This pattern is

unsurprising, given the much-publicized threat and global declines that these habitats are facing due to human activities, e.g. an estimated 85% decline in bivalve habitats worldwide (Beck et al., 2011). The loss of geogenic reefs has received less attention, as boulder excavations are likely a historic stressor unique to the Baltic Sea and few other regions. Still, these reef ecosystems are also threatened by beach nourishment activities that can burrow hard-bottom substrates (Kilfoyle et al., 2013), by damage inflicted from towed fishing gear (Kaiser et al., 2002; Boulcott & Howell, 2011) and by coastal infrastructure replacing subtidal boulder fields (Liversage & Chapman, 2018). Efforts to restore geogenic reefs have to date been scarce and current knowledge on effective methodologies is therefore very limited (Støttrup et al., 2017). Apart from constituting the main foundation of reef ecosystems, the hard substrate offered by geogenic habitats may provide many different functions and services, and marine boulders are today increasingly being implemented in the field of ecological engineering, for example to diversify coastal armoring structures such as seawalls and riprap (Liversage & Chapman, 2018). In addition, since boulders often co-occur with bivalve species (e.g. shellfish encrusting boulder surfaces; Green & Crowe, 2013), there has been growing interest in combining biogenic and geogenic restoration efforts (i.e. multi-habitat restoration) with potential mutual benefits for both reef types (Liversage, 2020). For example, boulder habitat may facilitate the recruitment of oyster larvae by providing hard substrate for settlement and a refuge from hydrodynamic forces such as high current velocity that could dislodge settled larvae (Whitman & Reidenbach, 2012; Liversage, 2020). Conversely, vertically protruding shellfish species could enhance the structural complexity within boulder reefs and supply food items for reef-associated taxa, while providing the additional service of water filtration that is particularly valuable in highly eutrophicated systems (Gren, Lindahl, & Lindqvist, 2009; Liversage, 2020). This increasing interest from various fields in the deployment or restoration of geogenic habitats highlights a need for conducting more empirical studies to better understand the effects on associated marine communities and to identify effective restoration strategies.

One of the few large-scale efforts on temperate reef restoration to date is the Blue Reef project, which aimed at rebuilding marine cavernous boulder reefs in the Kattegat between Denmark and Sweden (Støttrup et al., 2017). The project was initiated in 2007 and boulders were subsequently deployed in 2008 with the specific goal to stabilize the uppermost part of the reef and to restore crevices and a diverse topography of varying heights and inclinations (Stenberg et al., 2013; Støttrup et al., 2017). Given the large-scaled efforts, with 100,000 tons of large boulders deployed over approximately 27,400 m^2 of seabed (Stenberg et al., 2013), this project highlighted the importance of geotechnical baseline surveys to confirm the ability of the seabed to withhold the added weight and prevent partial sinking of the reef through the sediment (Støttrup et al., 2017). In addition, hydrodynamic modelling ensured that the restoration efforts did not negatively affect hydrographic conditions (i.e. currents and sediment transport) for nearby coastal areas and protected habitats (Støttrup et al., 2017). Biological monitoring was carried out before and after the reef restoration and revealed an estimated post-restoration gain in dry ash free biomass of 3 tons for benthic fauna and over 6 tons for macroalgal vegetation (Stenberg et al., 2013).

Fishing surveys revealed a significant increase in catches of commercially important cod (*Gadus morhua*) and saithe (*Pollachius virens*) after the restoration (Støttrup et al., 2014), while acoustic monitoring confirmed prolonged visitation rates to the reefs for both cod (Kristensen et al., 2017) and a regional apex predator (harbour porpoise *Phocoena phocoena*; Mikkelsen et al., 2013). This project showcases how a combination of careful planning in the initial and restoration stages, ongoing stakeholder involvement and consistent multi-year monitoring can result in the successful restoration of dynamic boulder habitats, while adding valuable insights into temporal restoration effects on associated marine communities. Unfortunately, multi-year monitoring using robust sampling designs has thus far proven to be the exception rather than the rule for marine habitat restoration (Jacob et al., 2018; Loch, Walters, & Cook, 2020).

1.4. Non-invasive monitoring of restored marine habitats

Monitoring programs constitute a crucial aspect of restoration projects by enabling an evaluation of the restoration techniques and the associated ecosystem responses. Monitoring efforts can thereby identify whether current actions are effective or need to be modified (i.e. adaptive management), answer specific questions of interest (e.g. which species or processes are returning to the ecosystem) and provide evidence to stakeholders that specific goals are being achieved (McDonald et al., 2016). For the marine environment, traditional survey methods that rely on catches from experimental fishing have been employed for many decades and allow for high replication under rigorous sampling designs (Murphy & Jenkins, 2010). However, the extractive nature of these catch-based methods makes them unsuitable for sampling within vulnerable areas, such as protected habitats or restoration sites, and while the surveys provide data on catchable species they offer no information on other species or habitats (Mallet & Pelletier, 2014). In addition, experimental trawling is destructive to benthic habitats and generally requires flat bottoms, while other fishing methods such as trapping and hook-and-line fishing have a high size-selectivity depending on trap and hook sizes (Murphy & Jenkins, 2010). As a non-invasive alternative, diver-based underwater visual census (UVC) has been used in shallow seas since the mid-1950s (Brock, 1954). While UVC can be effectively used to document spatio-temporal variations in relative fish abundance and species-habitat associations (SHAs), two major limitations include sampling biases induced by the reaction of fish to SCUBA divers (i.e. the 'diver effect'; Dickens et al., 2011) and the restricted depth at which UVC surveys can be conducted (Lowry et al., 2012).

1.4.1. Remote underwater video systems
The use of remote underwater video systems (RUVS) has been gaining traction since the mid-1990s and has now become a well-established technique to sample marine communities and habitats in a non-

invasive manner (Cappo et al., 2003). Accordingly, RUVS are employed in monitoring studies from the tropics to the polar regions and to survey marine habitats inaccessible to divers on SCUBA (e.g. due to sampling at great depth or in areas where large predators are present; (Cappo et al., 2011; Zintzen et al., 2012). Multiple video systems can be deployed consecutively in the field to enhance the spatial extent of the sampled area and make efficient use of time and resources (Langlois et al., 2012). RUVS provide a permanent record of the collected raw data that can be reviewed to minimize inter-observer variability (Cappo et al., 2003) and can be permanently archived or shared within repositories (e.g. GlobalArchive; https://globalarchive.org; Langlois et al., 2020). RUVS can be used to document species relative abundances, diversity indices and community compositions, as well as to identify behavioral aspects (e.g. foraging or spawning) and to describe the sampled habitat (e.g. percentage of reef coverage). In addition, the stereo-video adaptation of RUVS is useful to derive length-frequency distributions of fish and estimate the sampled biomass based on established length-weight relationships (Langlois et al., 2020). Relative abundances of species recorded by RUVS are commonly estimated using the MaxN metric, which represents the maximum number of individuals from a particular species seen in any of the video frames across the entire recording (Priede et al., 1994; Ellis & DeMartini, 1995; Cappo et al., 2003). The MaxN metric inherently underestimates true abundance, as separate visits by different individuals are not accounted for and only the visible portions of large schools are included in the MaxN count, and the metric is therefore considered a conservative estimate of true abundance (Cappo et al., 2003). At high fish densities, the MaxN metric may suffer from a saturation effect where large schools obscure additional individuals entering the frame, which again results in underestimation of true abundance through non-linearity with MaxN (Schobernd, Bacheler, & Conn, 2014). However, the only alternative metric proposed thus far, MeanCount (i.e. the mean number of individuals observed across a series of snapshots over a given interval), has been shown to over-inflate zero observations and to be less precise than MaxN (Campbell et al., 2015; Stobart et al., 2015). The MaxN metric therefore remains the most widely used (Whitmarsh, Fairweather, & Huveneers, 2017) and recommended standard metric for RUVS studies to date (Langlois et al., 2020).

RUVS generally consist of one (mono-RUVS) or two (stereo-RUVS) cameras mounted either on a heavy steel frame or on a similarly heavy platform from different material to ensure stability on the seabed. The RUVS setup used throughout this thesis consists of one GoPro camera (Hero 3 or 4), i.e. a mono-RUVS setup, attached to a steel pole and mounted on a heavy concrete tile, with the tile attached to a surface buoy via two ropes and an anchor for easy retrieval and redeployment from a small boat. While this method allows for recording the natural behavior of marine organisms unaffected by bait attractants (i.e. unbaited RUVS or UBRUVS), the system can be modified to present bait in the camera's field of view via a bait bag attached to a small pole (Fig. 1.5) and convert the system into baited RUVS (BRUVS). The core benefit and motivation for a majority of studies employing BRUVS monitoring is the
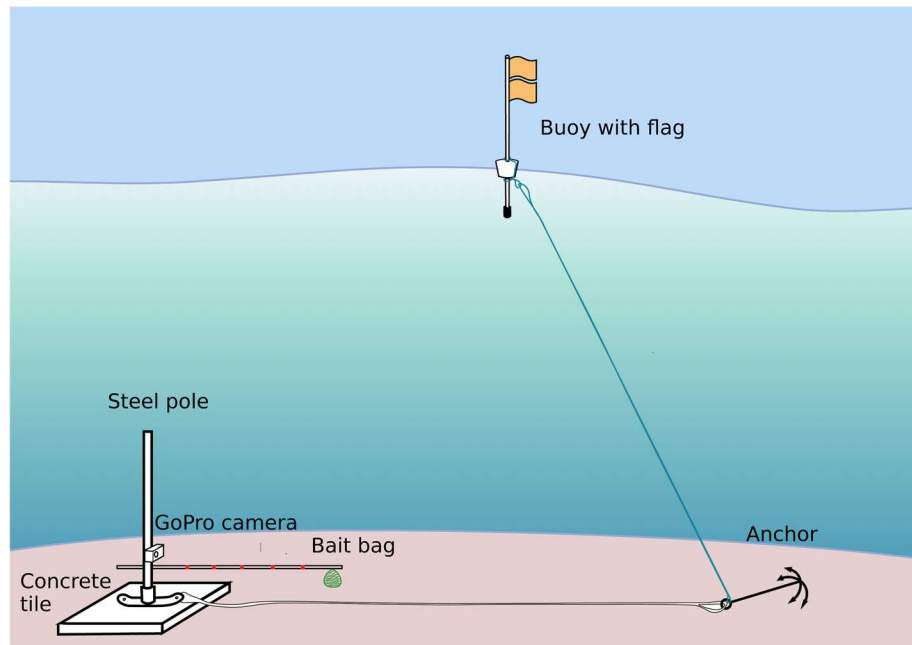
Figure 1.5 – Illustration showing a baited remote underwater video system (BRUVS) setup, typically consisting of a camera in underwater housing, attached to a steel pole and mounted on a heavy platform (here concrete tile) for stability on the seabed. A bait bag is attached via a bait arm extending horizontally in front of the camera, to attract marine organisms to the camera's field of view, while tape markers at known increments on the bait arm provide a scale for estimating distances away from the camera. The system is connected to a buoy via two ropes, facilitating easy retrieval and re-deployment from a small boat. Drawing of the camera unit by Elisenda Casabona Balcells. Created with BioRender (www.biorender.com).

method's ability to provide higher statistical power for inference through a reduced variability in the count data and fewer zero observations (Cappo et al., 2003; Bernard & Götz, 2012). While the larger area that is effectively sampled by attracting individuals to the camera could be regarded as another advantage (Cappo et al., 2003), the unknown dispersal of the bait plume in dynamic coastal waters implies that the exact sampled area remains unknown and that BRUVS monitoring is therefore unable to provide measures of absolute fish density (Colton & Swearer, 2010). Studies examining effects of the bait on the sampled community have thus far revealed increased efficiency of BRUVS in sampling demersal fishes relative to unbaited cameras, without reduced sampling efficiency for herbivores and omnivores (Harvey et al., 2007; Hannah & Blume, 2014). However, by attracting species from various habitats to the camera (e.g. reef fishes to sand habitats and vice versa), there remains a potential risk that BRUVS mask existing species-habitat associations (SHAs; Hannah & Blume, 2012), which has thus far not been tested empirically.

1.4.2. Environmental DNA

The ability to detect species from their DNA imprints left behind in aquatic environments, as first demonstrated by Ficetola et al. (2008) or for seawater samples by Thomsen et al. (2012), has ever since provided exciting new opportunities for monitoring marine communities without the need for direct observations. Environmental DNA (eDNA) is constantly shed by aquatic animals into the surrounding environment via scales, skin tissue, gametes, feces, metabolic waste or carcasses (Takahara et al., 2012; Thomsen & Willerslev, 2015). Collection and subsequent filtration of water samples in the field allows for capturing of such eDNA molecules, with the probability of DNA detection generally increasing with the water volume that is sampled (Hunter et al., 2019; Sepulveda et al., 2019). Following DNA extraction, species-specific detection can be obtained from the DNA material by using e.g. real time quantitative polymerase chain reaction (qPCR; Heid et al., 1996). Alternatively, community-wide assessments can be achieved using next generation sequencing (NGS) metabarcoding that utilizes primers targeting a broad taxonomic range (e.g. marine vertebrates; Sigsgaard et al., 2020). As marine eDNA research is still in its infancy, a number of significant challenges currently limit the adoption of this monitoring tool in decision-making frameworks within fields such as fisheries science and conservation (Darling & Mahon, 2011; Beng & Corlett, 2020). For example, DNA material may persist for extended periods (i.e. multiple days depending on the initial concentration; Collins et al., 2018) in the marine environment before degrading below detection thresholds, during which the material can potentially be transported far away from its initial source and be detected at sites where the target species is in fact absent (i.e. false positive detection; Darling & Mahon, 2011). Other challenges relate to differential rates of eDNA production (i.e. shedding) and degradation depending on biotic (e.g. body size and metabolic rate) and abiotic (e.g. temperature and pH) factors, as well as the current inability of eDNA monitoring to provide important ecological information such as life stage, condition (e.g. dead or living), sex ratio or reproductive behavior of target species (Hansen et al., 2018; Beng & Corlett, 2020). Nonetheless, given the rapid and low-cost nature of eDNA sampling combined with the potential to achieve high species detection rates, there is increasing interest from a conservation viewpoint to infer species abundances and ultimately population status from eDNA data. This potential prospect is therefore currently being evaluated at fast pace through numerous inter-method comparison studies, in which abundance measures from conventional methods (albeit with their own intrinsic biases) are tested for potential correlations with eDNA copy reads (e.g. Knudsen et al., 2019; Sato et al., 2021; Yates et al., 2021). A recent literature review found that an overwhelming 90% of studies reported on positive correlations between eDNA concentrations and abundance or biomass recorded by traditional methods (Rourke et al., 2021), although publication bias against negative results implies that the real percentage is likely substantially lower. A number of studies have thus far compared the performance of BRUVS and eDNA in biodiversity monitoring (Boussarie et al., 2018; Stat et al., 2019; Jeunen et al., 2020; Mercaldo-Allen et al., 2021) and generally demonstrate that a more holistic detection of the species assemblage is obtained when combining the two methods (though with eDNA detecting a broader range of taxa). However, despite the relative ease with which

(near-) concurrent sampling can be achieved from these methods in the field, there are to date no direct one-to-one comparisons of relative abundance recorded by BRUVS with species-specific qPCR detection from eDNA. This implies that the relationships between fish abundance observed on videos and eDNA concentration at the same locality remain poorly understood.

1.4.3. The importance of a robust sampling design

The formal definition of an ecological restoration activity, namely "*any activity whose aim it is to ultimately achieve ecosystem recovery, insofar as possible and relative to an appropriate local native model regardless of the period of time required to achieve the recovery outcome*" (McDonald et al., 2016), highlights two key features required for the evaluation of such activity. The first feature is the time aspect, which allows for expanding the most basic form of evaluating a restoration effort, i.e. an 'After design', in which the restored system is surveyed once after the restoration efforts took place. The 'After design' clearly suffers from a lack of reference to a pre-restoration condition, which can be remedied by adding a time dimension in the form of pre-restoration sampling, i.e. the 'Before-After' (BA) sampling design (Underwood, 1991; Smith, 2002). Although the BA design is conceptually the easiest way to assess the effect of any impact on a biological system, use of this design necessitates prior knowledge about the onset of an impact event to allow for pre- and post-impact assessments. While this does not pose a problem within the field of restoration ecology, i.e. restoration efforts are often carefully planned in advance and set to take place during a designated period, impacts that negatively affect ecosystems (e.g. chemical spills or wildfires) often arrive without warning. For such human-induced impacts, any 'before' data on the state of the system may not be available, although opportunistic sampling is sometimes possible (De Palma et al., 2018), e.g. when an ongoing forest monitoring study is interrupted by a wildfire. Nonetheless, inference based on a BA design is often confounded by coincidental natural events, such as extreme weather events or species turnover (Underwood, 1992; De Palma et al., 2018). Therefore, a lack of any apparent impact effect on the system evaluated under a BA design could still imply that the impact actually had an effect, e.g. if a natural deterioration in the system was halted by the impact event (Smith, 2002).

The second feature is the reference system (the "*appropriate local native model*"; McDonald et al., 2016), which if applied to the most basic 'After design' yields a comparison between a reference site and an impacted site, i.e. the 'Control-Impact' (CI) design. This design is the most cost- and time-efficient type of assessment, since sampling can be achieved within a single field season using consistent methods, and is therefore most frequently used in impact and post-restoration assessments (França et al., 2016; De Palma et al., 2018; Hale et al., 2019). The CI design is based on the assumption that the spatial reference site used facilitates appropriate inference about pre-impact conditions, also termed space-for-time (SFT) substitution (França et al., 2016). However, this assumption may often not be valid due to inherent temporal fluctuations of natural systems that are not being captured by the single-season

snapshot obtained from a CI design (Stewart-Oaten, Murdoch, & Parker, 1986; De Palma et al., 2018). A recent comparative study by França et al. (2016) found that the SFT approach greatly underestimated the impact of a logging operation on beetles in the Amazon, relative to a design that incorporated a temporal dimension to the sampling scheme. Yet, the ability to include temporal replicates may indeed be a luxury bestowed upon the field of restoration ecology (and a few others), while the majority of conservation studies examining the consequences of human activities will inevitably continue to rely on CI designs in the absence of pre-impact knowledge (Kibler, Tullos, & Kondolf, 2011; França et al., 2016). An important consideration when employing CI (or more advanced) designs is the type of control that should be designated. In restoration ecology, negative controls represent sites characterized by the pre-restoration degraded state, while positive controls (or target references) encapsulate the desired outcome through a functional reference ecosystem (Wortley, Hero, & Howes, 2013). Target reference sites are sometimes argued to be more useful in restoration ecology, since the goal is to converge the system towards a specific target condition and not merely diverge away from a degraded state (Wortley et al., 2013). However, there is currently little consistency in the type of reference site being used among restoration studies (Loch et al., 2020) and it is recommended that monitoring efforts should employ multiple reference types whenever feasible, to obtain a more comprehensive understanding of restoration effects (McDonald et al., 2016).

Merging the BA and CI designs results in a 'before-after control-impact' (BACI) design, which represents one of the most accurate and robust ways to estimate the true effect of an environmental impact (Stewart-Oaten et al., 1986; Christie et al., 2019). The main strength of a BACI design lies within the ability to disentangle spatial and temporal system fluctuations from the impact effect of interest, by comparing average differences between control and impact sites across time (i.e. before and after the impact event has taken place; Stewart-Oaten et al., 1986). However, it is relatively common for a single pair of control and impact sites to exhibit different temporal trajectories (e.g. due to population dynamics) unrelated to a particular impact event, and the use of replicate sites is therefore advocated to avoid such confounding effects (Underwood, 1994). While the use of multiple sites often results in an asymmetrical design (i.e. with a single impact site and multiple controls) for environmental impact assessments due to the impact event taking place at a single location (Underwood, 1992; Terlizzi et al., 2005), restoration studies may exploit the benefit of using multiple impact sites for more robust inference. Multiple control and impact sites can be paired and assessed through time series (i.e. 'before-after control-impact paired series' or BACIPS), taking account of inherent differences between control and impact sites and of temporal effects occurring on large spatial scales that affect both sites simultaneously (Bence, Stewart-Oaten, & Schroeter, 1996; Moland et al., 2013). Studies using BACI-type designs often employ statistical models in which time (before-after) and treatment (control-impact) are defined as fixed effects (e.g. Stenberg et al., 2015; Mills, Hamer, & Quinn, 2017) and the interaction between the two is tested for significance to provide evidence (or a lack thereof) for an impact effect. Such effects are most appropriately analyzed using Generalized Linear Mixed Models (GLMMs) that take account of hierarchies

in the sampling design in a flexible way (e.g. through random effects or grouping levels; Fisher et al., 2019). However, relying solely on measures of significance (e.g. p-values < 0.05) provides only limited information on the nature of the BACI effect (Murtaugh, 2002; Chevalier, Russell, & Knape, 2019), as significant positive interactions could have different underlying ecological implications (Fig. 1.6). Instead, BACI analyses should be supplemented with graphical interpretation of contrasts or additional metrics (e.g. 'CI-contribution' or 'CI-divergence'; Chevalier et al., 2019) that yield improved ecological insights. Alternatively, Bayesian statistics may offer a more intuitive probabilistic interpretation that could be particularly useful for restoration ecology and similar fields assessing impact events. For example, probabilities obtained from Markov Chain Monte Carlo (MCMC) sampling and BACI-ratio computation can readily be framed as evidence in favor of a particular effect size of interest (Conner et al., 2016), such as the probability that habitat restoration increased the abundance of a target species by at least 100% given the collected data and potential prior knowledge of the system. Such results can be easily conveyed to restoration managers and stakeholders relative to frequentist p-values that lack meaningful probabilistic interpretation (Ellison, 2004; Conner et al., 2016). Regardless of the statistical paradigm that one adheres to when conducting impact assessments, a BACI-type sampling design offers a powerful tool to disentangle true impact effects from natural fluctuations and ultimately facilitates robust inferences in ecological studies (Miller & Russ, 2014; Christie et al., 2019; Moland et al., 2021).
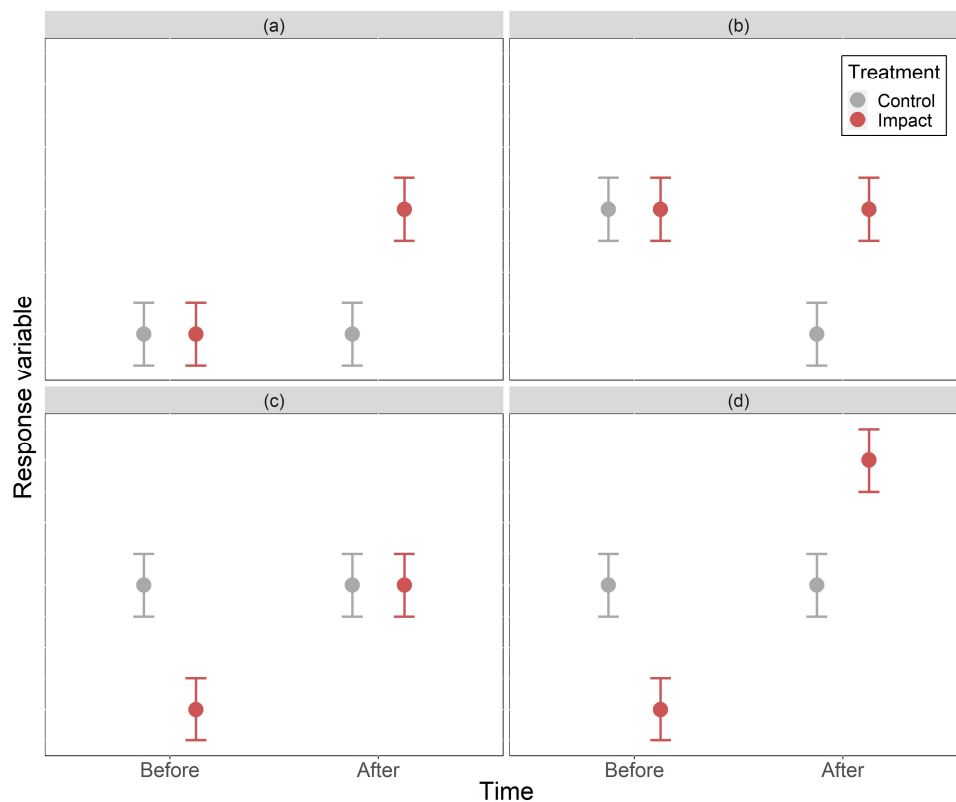
Figure 1.6 – Simplified example illustrating four scenarios in which a significant positive BACI interaction is detected given a single pre- and post-impact monitoring period, yet each with different underlying ecological implications. The

first scenario represents a classic textbook example of how an impact event (e.g. habitat restoration) positively affects the response variable relative to a negative control (e.g. the degraded state) (a). In the second scenario, a negative trend (e.g. a deteriorating condition of the system) is ameliorated by the impact event, for example following exclusion of a human-induced pressure through protected areas (b). The third and fourth scenario could be encountered when using target reference sites (or positive controls), as the impact site either converges towards the target reference (c) or potentially exceeds the target reference in performance, e.g. due to improved ecological conditions for a particular focal species (d).

1.5. Aims of the thesis

The overarching goal of this thesis is to advance the relatively young scientific discipline of marine habitat restoration by reporting on two small-scale efforts to restore geogenic reefs in the western Baltic Sea and by evaluating various non-invasive sampling techniques that facilitate monitoring of vulnerable habitats. Specifically, we restored boulder reef habitats in an area where natural reefs were historically present but entirely removed by extractive activities as indicated by archived nautical maps. We aimed to recover this important habitat type for reef-associated species in the study area (e.g. Baltic cod), while simultaneously testing the effect of spatial reef configuration on colonizing taxa by comparing restoration effects between different reef designs (Chapter 3). In addition, we investigate how the use of bait attractant in remote underwater video systems may affect the ability of this widely used sampling technique to accurately capture associations of marine taxa with coastal habitats. To this end, we perform a comparative assessment between species-habitat associations for seven common Baltic species as documented by baited and unbaited video systems (Chapter 4). Next, we report on one of the few efforts to date to restore cobble reefs (constructed from rocks with diameters between ~ 6 and 30 cm), in which we aimed to assess the effects on local species diversity and abundance, as well as the potential use of the newly restored reefs by commercially important species (e.g. cod and herring). We restored small reef units that alternated in height to further investigate whether reefs protruding higher up the water column (constructed using more than double the volume of cobbles) hosted a more abundant or diverse community relative to low reefs (Chapter 5). Finally, we examine the potential of emerging environmental DNA (eDNA) sampling to detect associations of common fish species with their surrounding habitat in a coastal bay characterized by a mosaic of different habitat types. We select four target species based on varying home ranges and mobility levels, to assess how these factors might influence the ability of eDNA sampling to accurately detect variations in species occurrences on a fine spatial scale (Chapter 6).

The following chapter provides a synopsis of the studies conducted as part of this thesis and outlines the research questions, methodologies, results and interpretations presented in the studies. The synopsis is followed by the four research articles (either published, accepted for publication or in advanced preparation) in Chapters 3 – 6 and a general discussion in Chapter 7, which synthesizes the

findings, evaluates the monitoring methods and discusses a number of applications for which results from this thesis are highly relevant.

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Chapter 2

Synopsis

2.1. Relevance of the SLOSS concept for marine habitat restoration

There has been a long-standing debate in conservation biology centered around the question whether Single Large or Several Small (SLOSS) habitat configurations promote the inhabitant species community in terms of the number of species (i.e. richness). The SLOSS debate emerged from equilibrium theory in island biogeography (MacArthur & Wilson, 1963), which formulates a relationship between immigration and extinction of species within a system and involves critical components such as species-area relationships, island-mainland dynamics and dispersal mechanisms (Yu & Lei, 2001). The concept has been extensively discussed and tested for a wide range of terrestrial systems (Volenec & Dobson, 2020). Studies thus far have indicated that large contiguous systems generally provide stable conditions for large population sizes, while several small habitats often possess a higher degree of habitat heterogeneity by encompassing a wider geographic area (Rösch et al., 2015; Volenec & Dobson, 2020). However, the concept has received less attention for aquatic systems and given the inherently dynamic nature of these systems (e.g. with relatively large dispersal distances; Carr et al., 2003), it is unlikely that theories and findings from terrestrial systems can be directly extrapolated to explain dynamics within aquatic environments. Furthermore, there remains an understudied potential to synergize findings from SLOSS studies with the field of restoration ecology (Bell, Fonseca, & Motten, 1997; Metzger & Brancalion, 2017). If restoration outcomes can be optimized by manipulating the spatial design of restored habitats, e.g. by restoring several small reef patches instead of one large reef, this could provide pertinent information for restoration managers aiming to effectively allocate limited resources, as well as for the field of restoration ecology in general.

In Chapter 3, we report on the results of a boulder reef restoration project carried out in a coastal area of the western Baltic Sea (WBS) that has been subjected to decade-long boulder extractions. We restored reefs in a single large (SL) and several small (SS) configuration to investigate the importance of SLOSS for marine habitat restoration. In addition, restoration effects were assessed for prominent focal taxa in the area, e.g. gadoids (including commercially important Atlantic cod and saithe) for which regional catches have consistently declined as a result of various anthropogenic stressors (potentially including historical boulder extractions in the study area). The restored reefs were monitored using remote underwater video systems (RUVS) using a before-after control-impact (BACI) sampling design. Our field monitoring efforts revealed that the restoration of boulder reefs within the study area had strong positive effects on a number of focal taxa, including gadoids, labrids and gobies. In particular, the restoration resulted in a 60-fold and 129-fold increase in gadoid abundance relative to reference sites, for

SL and SS reefs respectively. While reef design did not have an effect on species diversity (here expressed as species richness in accordance with the SLOSS concept), we found strong taxon-specific preferences with small-bodied mesopredators being highly associated with SS reefs. Further, the community composition at restoration sites deviated from reference sites after boulder habitats were restored, mostly driven by large-bodied piscivores (*Gadidae* spp.; *Ammodytidae* spp.). Distinct compositions were found between SL and SS reefs, with a number of small-bodied mesopredators associated with SS reefs. These results primarily demonstrate that boulder reef restoration is a promising tool to help recover declining gadoid populations in the WBS. Management interventions to recover predatory fish stocks are deemed crucial, as regional declines in large predatory fishes are negatively impacting coastal areas in the Baltic Sea through cascading top-down effects (Eriksson et al., 2011; Östman et al., 2016). The strong association of small-bodied mesopredators with SS reefs further highlights that the SLOSS concept has important implications for restoration ecology, as a community dominated by mesopredators may exert negative impacts on macroalgal communities through heavy predation on mesograzers (Östman et al., 2016) and thereby compromise the long-term resilience of restored habitats. Collectively, Chapter 3 demonstrates the importance of continued conservation and restoration of boulder habitats in the WBS and argues for a wider inclusion of concepts from landscape ecology and reserve design within restoration strategies.

While the collection of thousands of hourly video samples in our study allowed for robust statistical inference on restoration effects, there is a potential to drastically improve the efficiency of underwater video sampling both in terms of costs and time investment through the addition of bait (i.e. baited remote underwater video systems; BRUVS), which is further investigated in Chapter 4.

2.2. An assessment of the bait effect on species-habitat associations inferred from BRUVS

Underwater video sampling is increasingly used to monitor marine ecosystems, with stationary RUVS constituting one of the most well-established methods. RUVS can be deployed either baited (BRUVS) or unbaited (UBRUVS) and allow for recording of diversity and abundance metrics, as well as other valuable information such as feeding and reproductive behavior, predator-prey dynamics and abiotic parameters to describe the sampled habitat (Moore, Van Niel, & Harvey, 2011; Kelaher et al., 2015; McNeil et al., 2016). Recording species abundances in a variety of marine habitats allows for inference on the association between species and surrounding habitat, also known as species-habitat associations (SHAs). The use of BRUVS is very efficient at sampling demersal fish species, in particular predatory fishes, without compromising sampling efficiency of herbivores and omnivores (Harvey et al., 2007; Hannah & Blume, 2014). As individuals are attracted to the camera's field of view (FOV), this greatly reduces the variability of species counts between camera deployments and thereby increases the statistical power of inference relative to UBRUVS (Bernard & Götz, 2012). However, there has been some concern related to the ability of BRUVS to accurately document SHAs, since the attraction of species to the FOV could obscure their natural association with the surrounding habitat (Hannah & Blume, 2012).

In Chapter 4, we conduct a comparative assessment between BRUVS and UBRUVS to examine the ability of both methods to record SHAs for seven marine species in the western Baltic Sea. We selected the most common species in the area to ensure adequate data availability, which included Atlantic cod (*Gadus morhua*), right-eyed flatfish (*Pleuronectidae* spp.), Atlantic herring (*Clupea harengus*), whiting (*Merlangius merlangus*), goldsinny wrasse (*Ctenolabrus rupestris*), two-spotted goby (*Gobiusculus flavescens*) and shore crab (*Carcinus maenas*). We aimed to investigate whether BRUVS potentially obscure SHAs, by recording species abundance across habitats varying in cobble coverage from 0% to 100% and by comparing results from BRUVS and UBRUVS to SHAs documented in the literature. Our results revealed that all focal species responded to changes of cobble coverage on the seabed, implying that anthropogenic activities that modify benthic habitats (e.g. extraction of hard substrates) are likely to affect the abundance of a wide variety of marine taxa. UBRUVS documented significant negative associations with cobble for flatfish and shore crab, and significant positive associations for goldsinny wrasse and two-spotted goby, all in agreement with documented SHAs in the literature. However, BRUVS revealed some superiority by detecting associations for cod (unimodal) and whiting (negative) that were not recorded by UBRUVS, in addition to the SHAs described above. The detection of a unimodal relationship for cod, with abundance peaking at ~ 65-70% cobble coverage, provides new insights adding to the positive association described in literature. This result suggests that cod may prefer hard-bottom habitat of intermediate reef coverage (e.g. reef edges or mosaics), potentially allowing cod to include soft-bottom areas for foraging while remaining in close proximity to shelter offered by reef structures. Our study provides evidence supporting the use of BRUVS to document SHAs in coastal marine ecosystems. Documenting the association of coastal species with their surrounding environment is crucial to better inform management strategies aiming to protect ecologically important habitats.

2.3. The importance of cobble habitat in a degraded coastal system

The extraction of large marine boulders from the seabed has degraded geogenic reef ecosystems in the Baltic Sea for more than a century, until Danish law banned the extractive activities in 2010. However, rocks of smaller size (e.g. pebbles and cobbles) were not included in the legislation and are currently still being removed during various maritime activities (e.g. mussel dredging; Dolmer, 2002; Nielsen et al., 2020). These ongoing extractions have the potential to significantly affect local marine life, yet the topic has received little attention in Denmark. In Chapter 5, we report on one of the few efforts carried out to date to restore cobble reef habitats (consisting of rocks with a diameter between ~ 6 and 30 cm). Restoration effects were assessed via a comparison with negative controls (soft-bottom habitat) and target reference sites (i.e. natural reefs) using a before-after control-impact (BACI) design. We hypothesized that the restored cobble reefs would benefit local biodiversity and provide suitable habitat for a range of different marine organisms. For example, small-bodied reef taxa may find refuge within the small interstices created by the positioning of cobbles, while herring are known to deposit eggs onto

macroalgae growing on rocky surfaces (Aneer et al., 1983; Kanstinger et al., 2018), which in turn may attract various predatory species. In addition, cobble reefs were restored as 20 small reef units (truncated pyramids) that alternated in height between low (0.6 m) and high (1.3 m) reefs, which enabled us to investigate potential effects of reef height on early colonization of marine organisms. We hypothesized that the development of macroalgal communities would be enhanced at high reef units as a result of elevated light intensities reaching the top layers relative to low reefs, which was expected to induce differences in the composition of associated marine taxa.

Pre- and post-restoration monitoring using BRUVS (during April-May of 2017 and 2018, respectively) revealed a general decline in species diversity between the two periods across natural reefs and soft-bottom habitats. However, we found evidence that the restored reefs partly alleviated species diversity declines (for two out of three diversity metrics investigated), relative to the reference areas. Restored cobble reefs hosted a unique species assemblage that diverged away from soft-bottom habitat and slightly toward (yet distinct from) the composition at natural reefs. Notably, the availability of cobble substrate on the seabed was found to drive a large number of positive and negative correlations among marine species, with only a few residual correlations unexplained by seabed cobble coverage. Herring abundance on videos was unaffected by the restoration efforts and we found no evidence in support of herring spawning or reproductive behavior (e.g. individuals darting vertically through the water column; Aneer et al., 1983). Given that adult herring were seemingly ripe for spawning during the study period (Fig. 2.1), this may suggest that herring could be spawning at night (as we only sampled during daylight hours) or at different depths than investigated in our study (between 6 – 7 m). Reef height had no effect on the community composition of early-colonizing species, yet we note that the macroalgal community on the new reefs was still underdeveloped and characterized mainly by short-living ephemeral algae of low structural complexity. Since low reef units were constructed out of ~ 40 m^3 of cobbles relative to a volume

Figure 2.1 – Atlantic herring (*Clupea harengus*) caught in the study area of Sønderborg Bay during spring time, with the large-sized gonads suggesting that herring were ripe for spawning. Picture: Jon C. Svendsen.

of ~ 87 m³ for high reefs, long-term reef dynamics at the two designs (given adequate time for development of perennial macroalgae) could have important implications for future reef restoration efforts seeking to most efficiently allocate limited resources. We therefore aim to investigate the long-term effect of reef height in future studies based on ongoing post-restoration monitoring of the reefs. This study demonstrates that the restoration of cobble reefs can benefit species diversity and that cobble substrate is an important driver of species correlations within the study area of the western Baltic Sea. These results call for improved management actions to conserve cobble habitats given the ongoing extraction of small rocks from Danish waters.

Combined, results from Chapter 4 & 5 indicate that BRUVS can be successfully employed to infer species-habitat associations and that the inclusion of bait does not obscure distinct species assemblages (i.e. within sand habitats, natural reefs and restored reefs) on a small spatial scale. This opens up an opportunity to compare the relatively well-established BRUVS technique with a promising and fast emerging sampling method, environmental DNA (eDNA), and to assess how both these non-invasive techniques document habitat associations for coastal marine species. We pursue this aim in Chapter 6.

2.4. Combined use of baited video and eDNA to document fine-scale species-habitat associations
Global conservation efforts aiming to preserve coastal marine habitats demand accurate scientific knowledge on spatio-temporal distributions of inhabitant species. While BRUVS monitoring constitutes a well-established non-invasive technique to sample marine communities (Langlois et al., 2020), the collection and analysis of BRUVS data is still a relatively costly and time-demanding procedure. Recent advances in the field of environmental DNA (eDNA) have enabled the detection of marine organisms from DNA molecules in seawater (Thomsen et al., 2012). Marine organisms shed DNA into their surrounding environment via e.g. skin tissue, feces or gametes, and the DNA can be collected using relatively simple and low-cost sampling and filtration of seawater. Subsequent analysis in the laboratory can either focus on species-specific detection using quantitative polymerase chain reaction (qPCR) methods (Heid et al., 1996), or aim to delineate entire species communities from metabarcoding approaches (e.g. next-generation sequencing; NGS; Miya, 2021). eDNA sampling has a number of important advantages relative to traditional surveys, including the ability to rapidly obtain large sample sizes at a low cost, a high sensitivity that enables the detection of elusive species, and a reduced reliance on taxonomic expertise for species identification (Darling & Mahon, 2011; Boussarie et al., 2018; Alexander et al., 2020). However, as the amount of DNA available for detection depends on production, degradation and transportation rates (Hansen et al., 2018), there is immense variability in the spatiotemporal distribution of eDNA molecules in the marine environment depending on a range of biotic and abiotic factors (Barnes et al., 2014; Jo et al., 2019). This currently challenges our ability to correlate eDNA concentrations with the abundance or biomass of marine species (Yates, Fraser, & Derry, 2019), and a wide range of

comparative studies are warranted to investigate these relationships for different study systems and organisms.
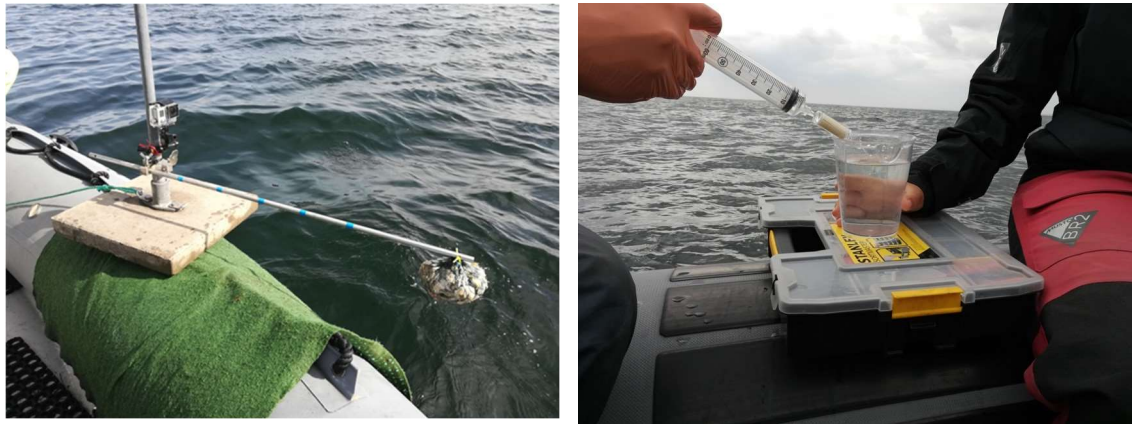
Figure 2.2 – Pictures of a BRUVS setup (left) and the procedure of sampling and filtrating seawater to detect eDNA molecules (right). We perform a direct comparison between these two non-invasive monitoring methods in Chapter 6, to assess their abilities to detect species-habitat associations (SHAs) on a fine spatial scale, as well as to examine potential correlations between fish counts from videos and eDNA concentrations in the seawater.

In Chapter 6, we conduct a comparative assessment between BRUVS and eDNA sampling to assess the ability of both methods to detect four common Baltic fish species (cod *Gadus morhua*; flounder *Platichthys flesus*; plaice *Pleuronectes platessa* and goldsinny wrasse *Ctenolabrus rupestris*) and to describe their associations with surrounding soft- and hard-bottom habitat. To this end, we filtered 41 seawater samples taken from April-June 2018 and paired each sample with two subsequently deployed BRUVS to facilitate a direct comparison. The study was conducted within a coastal bay characterized by a patchy distribution of coastal habitat, implying that soft-bottom was often in close proximity (i.e. within 100s of meters) from hard-bottom habitat and vice versa. Our aims in this study were three-fold: i) to evaluate the ability of both methods to detect SHAs on a fine spatial scale; ii) to compare species detection rates between the methods for all focal species and iii) to examine potential correlations between fish counts from videos and eDNA concentrations in the seawater. Focal species were selected based on their differential life style (resident vs transient) and mobility (sedentary vs highly mobile), to assess whether these factors had an effect on the observed patterns.

Results from eDNA sampling indicated that DNA concentrations were generally very low in our study area for all focal species. This necessitated a qualitative data treatment, since concentrations were found to be below the limit of quantification (LOQ) that represents a threshold above which concentrations can be quantified with adequate precision (Klymus et al., 2020). This motivated us to explore an additional semi-quantitative approach, in which we used the proportion of qPCR replicates that amplified

as a proxy for DNA concentrations present in the samples. Both our semi-quantitative and the qualitative approach provided strong evidence for a sand association for flounder and a reef association for wrasse, in accordance with patterns observed in BRUVS data. However, BRUVS detected an additional reef association for cod and sand association for plaice, which were not apparent in the eDNA data. Still, eDNA sampling outperformed BRUVS in terms of detection rates for three out of four species, including conspicuously large and mobile cod which are easily detected on BRUVS recordings, while detection rates for wrasse were similar between the methods. Combined, the very high residency of wrasse (Halvorsen et al., 2021), the equal detection rates between methods and the strong reef-association detected using eDNA sampling suggests that eDNA traces of wrasse were highly localized, most likely due to rapid dilution of DNA material below detectable levels away from the source. Similarly, the strong evidence of a sand association for flounder also suggests that flounder eDNA traces were localized, possibly resulting from their sedentary and cryptic lifestyle, although we did not find the same evidence for plaice which exhibits a very similar lifestyle. Plaice generally showed the lowest DNA concentrations of all focal species, yielding many 'non-detections' that likely resulted in low statistical power, while the higher resolution of BRUVS data (using an abundance metric) allowed for the detection of a sand association, despite a lower detection rate of plaice relative to eDNA. Finally, although there was some evidence indicating a positive correlation between wrasse abundance on videos and eDNA detection, we did not find sufficient evidence for the remaining species and the explanatory power of the relationships was generally low. This highlights the ongoing challenge of inferring fish abundance from eDNA signals in dynamic marine systems. Our study demonstrates that eDNA sampling is capable of detecting fine-scale SHAs for coastal marine species, yet this ability likely depends on the lifestyle of the study organism in addition to factors generally known to govern eDNA concentrations in the marine environment (i.e. production, degradation and transport; Hansen et al., 2018). Future studies aiming to describe relationships between marine species and benthic habitats may benefit from high detection rates of eDNA sampling, yet such studies should critically consider the lifestyle of the study organism and how this may affect the spatial explicitness of eDNA data. Combined use of eDNA with traditional survey methods is advised to shed further light on such potential relationships. We further recommend that studies sample large water volumes (> 1L) to increase the potential for detecting adequate eDNA concentrations for quantitative assessments.

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Chapter 3

Restoring marine ecosystems: spatial reef configuration triggers taxon-specific responses among early colonizers*

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Abstract

1. The longstanding debate in conservation biology on the importance of single large or several small (SLOSS) habitats for preserving biodiversity remains highly relevant, given the ongoing degradation and loss of natural habitats worldwide. Restoration efforts are often constrained by limited resources, and insights from SLOSS studies therefore have important implications if restoration efforts can be optimized by manipulating the spatial configuration of restored habitats. Yet, the relevance of SLOSS for habitat restoration remains largely unexplored.

2. Here, we report the effects of spatial reef configuration on early colonization of marine organisms after restoring boulder reef habitats. Reefs were restored in single large (SL) and several small (SS) designs in the western Baltic Sea, where century-long boulder extraction has severely degraded large reef areas and likely exacerbated regional declines in commercially important gadoids (*Gadidae spp.*). We sampled the field sites using remote underwater video systems in a before-after control-impact (BACI) design and obtained probabilistic inferences on restoration and SLOSS effects from Bayesian hierarchical models.

3. Probabilities of a positive restoration effect were high (>95%) for gadoids, labrids and demersal gobies, moderate (60-75%) for species richness and sand gobies, and low (<5%) for flatfish abundance. Notably, gadoid abundance increased 60-fold and 129-fold on average at SL and SS, respectively. The species composition at restored reefs deviated from control sites, mainly driven by large-bodied piscivores.

4. Spatial reef configuration had the strongest effect on small-bodied mesopredators, including gobies, which were more abundant at SS and driving distinct species assemblages between the reef designs. In addition to providing suitable conditions for reef species, results suggest that SS can also benefit soft-bottom taxa, possibly through a dispersed predator-mediated effect relative to SL.

5. *Synthesis and applications.* This study demonstrates that boulder reef restoration can strongly promote the abundance of exploited gadoids (e.g. Atlantic cod) and is therefore a promising management tool to support top-down controls by predatory fishes in degraded marine systems. The higher abundance of mesopredators at reefs with a 'several small' configuration suggests that the 'Single Large or Several Small' (SLOSS) dilemma could have long-term implications for trophic structure and resilience of restored habitats, and should therefore become an important facet within restoration strategies.

Resumé

1. I forbindelse med beskyttelse af dyrs og planters levesteder har man i lang tid diskuteret, om det er bedst at beskytte ét stort område eller mange små områder (ESEMS-diskussionen).

Spørgsmålet er stadigvæk relevant, fordi levesteder fortsat går tabt mange steder i verden. Levestederne kan restaureres, men projekterne har ofte begrænsede midler til rådighed. Viden fra ESEMS-undersøgelser er derfor vigtig, fordi resultaterne fra restaureringsprojekter formodentligt bliver bedre, hvis den rummelige udbredelse af de restaurerede levesteder optimeres i forhold til ESEMS. Alligevel anvendes viden fra ESEMS-undersøgelser sjældent i restaureringsprojekter.

2. I nærværende studie rapporterer vi effekterne af forskellige rummelige udbredelser af restaurerede stenrev i forhold til kolonisering af marine organismer. Stenrevene blev enten restaureret som enkelte store (ES) stenrev eller som mange små (MS) stenrev. Projektet blev udført ved Sønderborg Bugt, hvor opfiskning af sten har fundet sted igennem årtier, hvilket har reduceret antallet af naturligt forekommende stenrev. De manglende stenrev betyder bl.a., at torskefisk (*Gadidae spp.*) har færre levesteder. Vi brugte undervandskameraer til at undersøge kontrolområder og kommende revområder både før og efter udlægning af stenrev. Data fra videooptagelserne blev anvendt til at estimere effekterne af de udlagte stenrev.

3. Analyserne viste, at sandsynligheden for en positiv restaureringseffekt var høj (> 95%) for torskefisk, læbefisk og bundlevende kutlinger, moderat (60-75%) for artsrigdommen og sandkutlinger, og lav (<5%) for fladfisk. Gennemsnitligt steg forekomsten af torskefisk 60 gange ved ES stenrev og 129 gange ved MS stenrev.

4. Det var især smådyr som kutlinger, der var påvirkede af forskellighederne i stenrevenes rummelige udbredelse. Der var højere forekomst af smådyrene ved MS stenrev, hvor der ligeledes var en særlig artssammensætning af smådyrene. Resultaterne viser, at restaurerede stenrev giver levesteder til en række rev-arter, der formodentligt drager nytte af nærliggende blødbundsområder i forbindelse med MS stenrevene.

5. *Syntese og anvendelse*. Vores undersøgelse viser, at restaurering af stenrev i høj grad kan forøge forekomsterne af torskefisk. Restaurering af stenrev er derfor et lovende værktøj til at genskabe tidligere forekomster af rovfisk som torsk, der kan påvirke havets fødekæder og økosystemer positivt ved at æde en række mindre organismer. De højere forekomster af smådyr som kutlinger ved MS stenrevene indikerer, at ESEMS overvejelser kan have langsigtede konsekvenser for fødekæder og økosystemers modstandsdygtighed ved restaurerede levesteder. Derfor bør ESEMS overvejelser integreres i fremtidige restaureringsstrategier.

3.1. Introduction

Coastal waters around the globe are facing cumulative impacts from human-induced stressors at an accelerating pace (Halpern et al., 2019). In the Baltic Sea, a combination of resource extraction, eutrophication and pollution has severely degraded coastal habitats (Korpinen, Meski, Andersen, & Laamanen, 2012). The seabed of the Baltic Sea is generally dominated by mud and sandy-mud of smooth topography, yet large boulders are scattered across the low relief areas as a result of glacial erosion and deposition (Beisiegel, Tauber, Gogina, Zettler, & Darr, 2019; Kaskela & Kotilainen, 2017). Accumulations of boulders locally form geogenic reef systems, providing hard substrate and structural complexity in an otherwise homogenous environment. The hard substrate enables anchorage of macroalgae and sessile fauna, and complex reef structures such as holes, crevices and ledges offer shelter for numerous marine species. In recent history, extraction of marine boulders for the construction of harbors, jetties and other coastal structures has reduced the quality and volume of hard bottom substrate in the Baltic Sea (Dahl, Lundsteen, & Helmig, 2003). While boulder extraction from Danish waters was prohibited in 2010 (Kristensen et al., 2017), the permanent removal of hard substrate constitutes a fundamental shift in reef availability, hampering recovery of associated flora and fauna and making habitat restoration efforts warranted (Johnson, Chabot, Marzloff, & Wotherspoon, 2017).

A longstanding conundrum within the field of conservation biology involves the efficacy of a single large or several small (SLOSS) habitat configuration in promoting species richness (Cole, 1981; Diamond, 1975; Simberloff & Abele, 1982). Notably, the emergence of SLOSS from equilibrium theory in island biogeography (MacArthur & Wilson, 1963) resulted in numerous studies examining SLOSS for terrestrial systems (overview in Volenec & Dobson, 2020). Single large (SL) habitats tend to provide stable conditions for large population sizes, while several small (SS) systems often include more heterogeneous habitats and cover a greater geographical extent (Rösch, Tscharntke, Scherber, & Batáry, 2015; Volenec & Dobson, 2020). However, extrapolating empirical evidence from terrestrial SLOSS studies to marine systems may not be a valid approach, given the marked differences in physical, ecological and evolutionary processes between marine and terrestrial environments (Carr et al., 2003). For example, dispersal from marine reserves may replenish neighboring habitats and facilitate habitat connectivity across large distances (>40 km; Manel et al., 2019), whereas the productivity of terrestrial reserves highly depends upon local fecundity, often with limited exchange among populations (Carr et al., 2003). As such, SLOSS studies from a wide range of marine systems are warranted to assess potential effects on associated marine communities and elucidate the underpinning mechanisms. To date, studies examining SLOSS for marine environments have been limited to marine reserves (Fovargue, Bode, & Armsworth, 2018; Puckett & Eggleston, 2016; Stockhausen & Lipcius, 2001), coral reefs (Acosta & Robertson, 2002), concrete modules (Bohnsack, Harper, McClellan, & Hulsbeck, 1994) and seagrass beds (McNeill & Fairweather, 1993). While investigated response variables differ between studies, the

majority of findings indicate SS > SL with only one study concluding SL > SS (Stockhausen & Lipcius, 2001) and one study finding no effect (Acosta & Robertson, 2002).

The SLOSS concept has received much attention in conservation biology, landscape ecology and reserve design, yet there remains a largely unexplored potential to synergize SLOSS theories and empirical evidence with restoration ecology (Bell, Fonseca, & Motten, 1997; Metzger & Brancalion, 2017). For example, identifying the appropriate spatial configuration of restored habitat may facilitate successful recruitment of fauna (Bell et al., 1997) and optimize restoration outcomes (Belder et al., 2019). Boulder reefs provide an ideal case study to test the importance of SLOSS for restoration ecology, as boulders represent patches of isolated habitats that are easily manipulated (e.g. in spatial configuration; Chapman, 2017). In addition, boulder substrate is increasingly incorporated in ecological engineering (Liversage & Chapman, 2018), for example to diversify seawalls (Chapman & Underwood, 2011), and in restoration efforts of geogenic (Kilfoyle, Freeman, Jordan, Quinn, & Spieler, 2013; Støttrup et al., 2017) or biogenic reefs (i.e. multi-habitat restoration; Liversage, 2020). Studies on boulder reefs have highlighted the importance of microhabitat (i.e. centimeters; Liversage, Cole, Coleman, & McQuaid, 2017) and geological features across large scales (i.e. kilometers; Beisiegel et al., 2018; Franz et al., 2021) in shaping reef community dynamics, but the effects of spatial reef configuration on intermediate scales (i.e. reef patches; 10–100 m) are still poorly understood. Accordingly, there is a need to examine the importance of SLOSS in shaping the marine community associated with boulder substrates and the underlying implications for restoration efforts in general.

In this study, we constructed SL and SS boulder reefs in a coastal area of the western Baltic Sea where reefs were depleted by historical extractive activities. We monitored the field sites using remote underwater video systems (RUVS) before and after the reef restoration to address three aims. First, we assessed the effects of reef restoration on species richness and relative abundance of prevalent taxa by comparing pre- and post-restoration sites. Second, we compared these metrics for SL and SS to address SLOSS in relation to marine habitat restoration. Finally, we delineated the species composition of pre- and post-restoration sites and assessed whether reef configuration affected the composition of colonizing species.

3.2. Materials and Methods

3.2.1 Study area & design

Our study was carried out within Flensborg Fjord, a Natura 2000 designated protected area between Denmark and Germany. We selected six field sites (Figure 3.1a) using archived information on historic boulder extraction sites (Mammen Kruse, 2016). All sites had a depth between 6 and 7 m and were

separated by at least 500 m. We used a before-after control-impact (BACI; Christie et al., 2019) sampling scheme, by assigning two control (sandy bottom) sites and four impact (restoration) sites for sampling before and after the reef restoration. Pre-restoration sampling took place on daily basis during the summer months of June and July 2016, as long as weather conditions allowed for safe boating
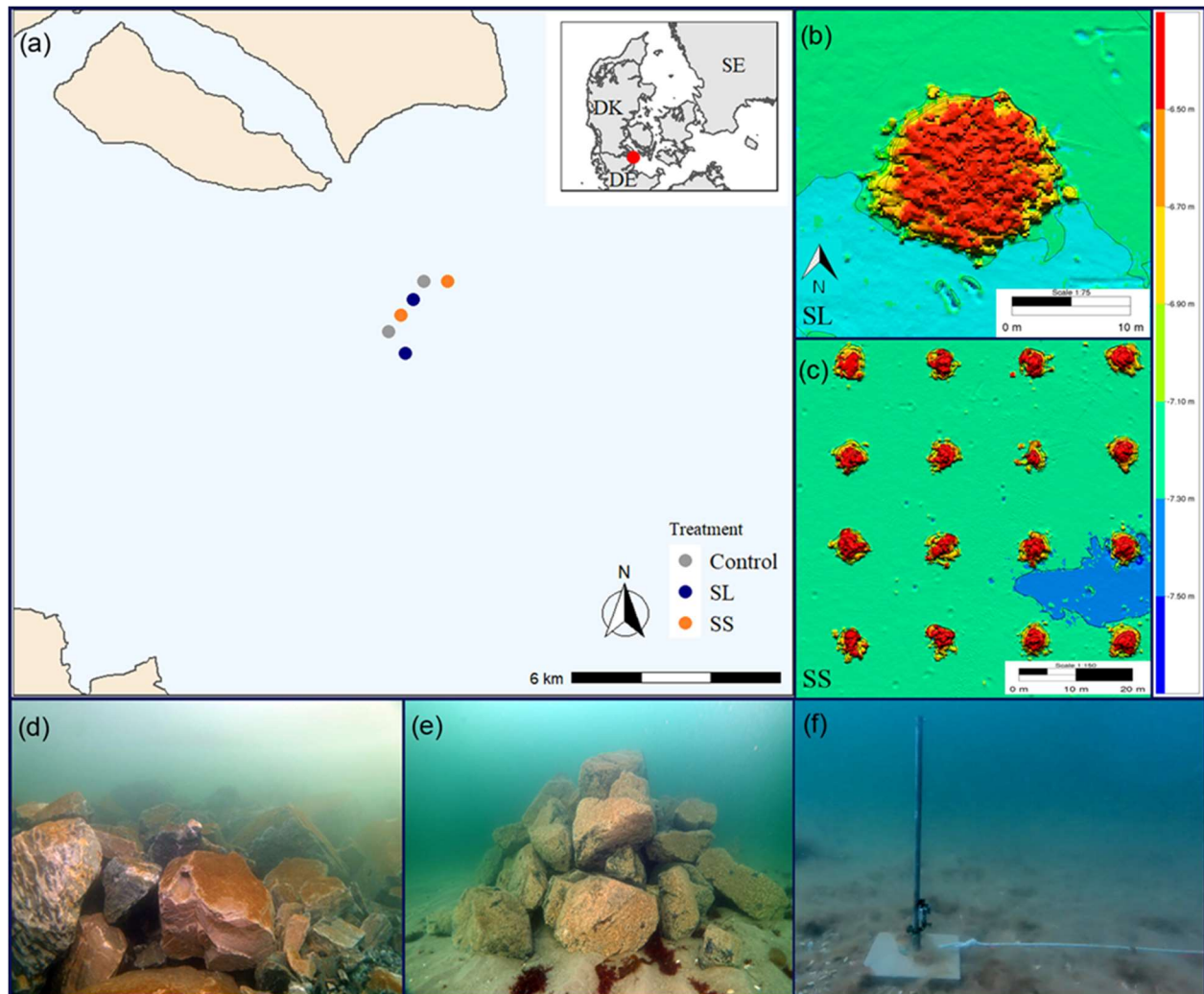
Figure 3.1 – Overview of the study location and spatial reef configurations. The map shows the study area in the southwestern Baltic Sea, with colors denoting locations of the treatment replicates (a). At two restoration sites, reefs were constructed as a single large (SL) reef (b, d; diameter = 13 m), while reefs were distributed in a 4x4 configuration of several small (SS) reefs (c, e; diameter = 3.5 m) at the two remaining restoration sites. Two soft-bottom sites served as controls. All sites were monitored using remote underwater video systems (f).

(details provided in S1 of Supporting Information). Subsequently, we constructed boulder reefs in December 2017 and January 2018 and conducted post-restoration sampling during June and July of 2018 (i.e. approximately 5-6 months after the restoration). All field sites therefore consisted of empty sand bottoms in 2016 but were still separated into control and restoration sites (Figure 3.1) to disentangle natural temporal fluctuations from the restoration effects.

Reefs were constructed using boulders with a diameter of 0.5-1.5 m, obtained from a quarry in Norway. The total amount of restored habitat at each of the four restoration sites was standardized at a reef volume of 500 m^3. The two remaining sites served as soft-bottom control sites (Figure 3.1a). At two restoration sites, the volume of boulders (500 m^3) was deposited at one location to form a single large (SL) reef (Figure 3.1b; 3.1d), whereas boulders were distributed in a 4x4 configuration at the remaining two restoration sites to construct several small (SS) reefs (Figure 3.1c; 3.1e). All constructed reefs were 2 m in height. The sampled area was standardized for each site at 3250 m^2, which encompassed the entire SS configuration (Figure 3.1c; including intermediate sand) and included a 25 m radius of sand habitat surrounding the SL reefs (Figure 3.1b).

3.2.2 Data collection

We deployed unbaited RUVS (Figure 3.1f) to monitor the field sites. We used GoPro cameras (Hero 3, 3+ and 4; GoPro, USA), equipped with a timer (Time Lapse Intervalometer or BlinkX; CamDo Solutions, Canada), which programmed the cameras to record for two minutes every hour. Each deployment consisted of 24-60 two-minute recordings (i.e. recordings across 24-60 hours), which were used as individual sampling units. We deployed a maximum of 3-4 RUVS at the same field site on a given sampling day, with distances between the RUVS actively maximized within the confines of the sites. We used a side scanner (Lowrance Elite-7 Ti; Lowrance Electronics, USA) to scan the bottom for the desired substrate (i.e. restored reef or sand). Further details on sampling procedures are provided in the Supporting Information.

3.2.3 Video analyses

We analyzed video recordings using VLC Media Player (VideoLan; www.videolan.org). Relative abundance of species was measured as MaxN counts (Cappo, Harvey, Malcolm, & Speare, 2003), a widely used metric obtained by counting the maximum number of individuals of a certain species in any video frame to avoid double-counting. Observers identified individuals to the lowest possible taxonomic category. In case species-level identification was unattainable, individuals were grouped by genus or family level. The sampled marine community included all conspicuous organisms within the field of view of the camera, from slow-moving benthos (e.g. starfish) to mobile pelagic species (e.g. herring; Table S3.2). However, sampling notably excluded sessile taxa (e.g. ascidians, barnacles and sponges) which

could not be easily identified and counted from the RUVS recordings and for which other video methods such as downward-facing towed cameras are more suitable (Beisiegel et al., 2018). While we did not identify macroalgae observed on the videos, both vegetation and boulder coverages were estimated as proportions of the total visible seabed within a video frame, to describe the sampled habitat (Figure S3.2). In addition, functional visibility was estimated for each hourly video sequence from rope markers deployed along the camera's field of view.

Table 3.1 – Overview of the sampling effort and descriptive statistics on the raw observations. The number of camera deployments and extracted two-minute hourly samples (excluding night recordings) is given for each of the six field sites (Figure 3.1), whereas descriptive statistics are pooled for each treatment (Control, SL, SS) and time (pre- and post-restoration) combination.

	Pre-restoration (2016)						Post-restoration (2018)					
Treatment	Control		SL		SS		Control		SL		SS	
Sampling effort (for two replicate field sites per treatment)												
Camera deployments	22	16	12	16	22	18	39	40	44	42	55	57
Two-minute samples (N)	529	341	315	270	367	365	1062	1086	1213	1093	1658	1650
Descriptive statistics: Mean count 2min.$^{-1}$ (SD)												
Species richness	1.81 (1.15)		1.83 (1.12)		1.88 (1.12)		2.31 (1.29)		2.31 (1.30)		2.77 (1.41)	
Gadoids	0.01 (0.10)		0.00 (0.06)		0.00 (0.04)		0.00 (0.06)		0.47 (0.87)		0.25 (0.58)	
Labrids	0.01 (0.13)		0.04 (0.22)		0.03 (0.19)		0.14 (0.42)		0.26 (0.61)		0.23 (0.53)	
Flatfishes	0.02 (0.16)		0.02 (0.15)		0.03 (0.17)		0.07 (0.29)		0.02 (0.14)		0.04 (0.21)	
Demersal gobies	0.30 (0.80)		0.21 (0.99)		0.03 (0.19)		2.37 (3.94)		2.39 (5.70)		2.81 (7.13)	
Sand gobies	0.80 (1.17)		1.23 (1.74)		1.63 (1.69)		0.43 (0.87)		0.17 (0.54)		0.70 (0.94)	

3.2.4 Statistical analysis

We used Bayesian hierarchical models to analyze the effects of reef restoration and configuration on species richness and abundance of prevalent marine taxa. Specifically, we fitted Generalized Linear Multilevel Models (GLMLMs) to assess efficacy of the restoration efforts given our BACI sampling design. The integer response variables were initially modelled using a Poisson distribution with a log-link and subsequently assessed for dispersion. Models showing overdispersion were improved using a negative binomial distribution (Figure S3.7), while the Conway-Maxwell Poisson distribution proved effective at dealing with underdispersion (Figure S3.8; Huang, 2017).

Similar to previous studies employing a BACI design (e.g. Stenberg et al., 2015), we focused on the interaction between time period (pre- and post-restoration) and treatment (Control, SL and SS) to disentangle restoration effects from temporal fluctuations in the system. Additional GLMLM covariates included vegetation coverage on the seabed and the natural logarithm of functional visibility (Zuur & Ieno, 2016). We defined group-level effects for each model to incorporate inherent variations due to sampling design. Specifically, varying intercepts at the site-level accounted for correlations between deployments from the same site, while varying intercepts for each deployment were nested within site to account for additional correlation between hourly samples from the same camera deployment. Hierarchical models assume that residuals are independent without spatial or temporal dependencies. Given our sampling method however, with hourly samples and multiple deployments per site, this may not be a valid assumption. We therefore tested all models for residual patterns and included a Gaussian process (exponentiated-quadratic kernel) on deployment coordinates and/or an autoregressive correlation structure on hourly samples to account for spatial- and temporal autocorrelation, respectively, if such dependencies were present. Prior predictive checks (Figure S3.4) were performed following Gabry et al. (2019). Finally, the most parsimonious model for each response variable (Table S3.3 & S3.4) was selected through leave-one-out cross-validation. This method provides estimates of out-of-sample prediction error using Pareto smoothed importance sampling (Vehtari, Gelman, & Gabry, 2017), allowing for comparison of predictive accuracy between multiple candidate models.

We followed the protocol outlined by Conner et al. (2016) and employed a combination of Bayesian Markov Chain Monte Carlo (MCMC) sampling and BACI ratio computation to estimate probabilities of different restoration effect sizes. First, we extracted posterior samples from all models for each time period and treatment level, with the number of samples (N) depending on the number of post-warmup iterations for each model (Table S3.3). Next, we computed "before-" and "after-ratios" as follows:

$$R_{i|\text{SL\_B}} = \frac{\hat{Y}_{i|\text{SL\_B}}}{\hat{Y}_{i|\text{Control\_B}}} \; ; \; R_{i|\text{SL\_A}} = \frac{\hat{Y}_{i|\text{SL\_A}}}{\hat{Y}_{i|\text{Control\_A}}} \; ; i = 1, \dots, N \tag{1}$$

with $R_{i|\text{SL\_B}}$ denoting the "before-ratio" and $R_{i|\text{SL\_A}}$ the "after-ratio" for the i-th iteration of the SL treatment, while $\hat{Y}_{i|\text{SL}}$ and $\hat{Y}_{i|\text{Control}}$ represent the fitted values (on the response scale) for the i-th iteration of the SL and control treatment, respectively. The same calculations were applied to the SS treatment. We then computed "BACI-ratios" for the two reef configurations:

$$R_{i|\text{SL\_BACI}} = \frac{R_{i|\text{SL\_A}}}{R_{i|\text{SL\_B}}} \; ; \; R_{i|\text{SS\_BACI}} = \frac{R_{i|\text{SS\_A}}}{R_{i|\text{SS\_B}}} \; ; i = 1, \dots, N \tag{2}$$

yielding a posterior distribution of N BACI-ratios for each reef treatment. Since a BACI ratio of 1 indicates an equal "before-" and "after-ratio", the posterior density (i.e. area under the curve) of ratios > 1 is equivalent to the probability of a positive restoration effect on the response variable. Therefore, this method allows for a straightforward probability assessment of any impact effect size of interest. Finally, we calculated "SLOSS-ratios" to compare restoration effects between the two reef configurations:

$$R_{i|\text{SLOSS}} = \frac{R_{i|\text{SS\_BACI}}}{R_{i|\text{SL\_BACI}}} \; ; \; i = 1, \dots, N \tag{3}$$

yielding a posterior distribution of N SLOSS-ratios with the density of ratios > 1 being equivalent to the probability of a larger response variable at SS.

To assess the effects of both reef restoration and configuration on species composition, we performed a Bayesian ordination and multivariate regression analysis. Specifically, we fitted pure latent variable models (LVMs) to run a model-based unconstrained ordination and visualize sites with indicator species in a low-dimensional plot (Hui, 2016). This approach allows for validation of distributional and residual assumptions, as well as a comparison of information criteria values between different models. We initially used a Poisson distribution to model the multivariate species abundances, but information criteria values and model validation plots (Figure S3.11) indicated that using a negative binomial distribution improved the fit. We excluded species occurring in less than three camera deployments and defined a site-level row effect to account for variations in abundance per site to focus on community composition. In case of community differences, we further tested the effect of environmental parameters (i.e. reef configuration and vegetation) by fitting correlated response models and inspecting residual correlations (Hui, 2016).

All analyses were performed in R statistical software version 3.6.1 (R Core Team, 2020). The Bayesian hierarchical models were fitted using the 'brm' function in the *brms* package (Bürkner, 2017), implementing Bayesian models through the R interface of the STAN programming language (Carpenter et al., 2017). Spatial and temporal autocorrelation tests were performed using the *DHARMa* package (Hartig, 2018) after converting the Bayesian model fits into DHARMa objects with the 'createDHARMa' function. Posterior predictive checks were performed with the *bayesplot* package (Gabry & Mahr, 2018). The LVMs were fitted using package *boral* version 1.8 (Hui, 2016).

3.3. Results

3.3.1 Sampling effort and observed community

Field sampling resulted in a total of 383 camera deployments of which 106 deployments were completed pre-restoration and 277 deployments post-restoration, yielding 9949 two-minute hourly video samples (Table 3.1). We identified 40 unique species, including 30 marine fishes, 9 invertebrates and 1 marine mammal, belonging to 23 families. During pre-restoration sampling, we recorded 28 unique species (20 species at control, 18 at SL and 20 at SS), while 33 species were recorded post-restoration (26 at control, 24 at SL and 31 at SS). We grouped MaxN counts of the most prevalent fish species on family or order level to model responses of these prominent taxa to the reef restoration. The five focal groups included gadoids (*Gadidae* spp.), which are mobile predatory fishes comprised mainly of economically important Atlantic cod (*Gadus morhua*) and saithe (*Pollachius virens*), as well as labrids (*Labridae* spp.; mostly goldsinny wrasse, *Ctenolabrus rupestris*), flatfishes (*Pleuronectiformes* spp.; including both left-eyed and right-eyed species), and gobies (*Gobiidae* spp.). Given the differences in habitat association within the goby family, we divided gobies into demersal gobies (including two-spotted goby, *Gobiusculus flavescens* and transparent goby, *Aphia minuta*) and sand gobies (*Pomatoschistus spp.* and black goby, *Gobius niger*). A complete overview of observed species and focal groups is provided in the Supporting Information (Table S3.2).
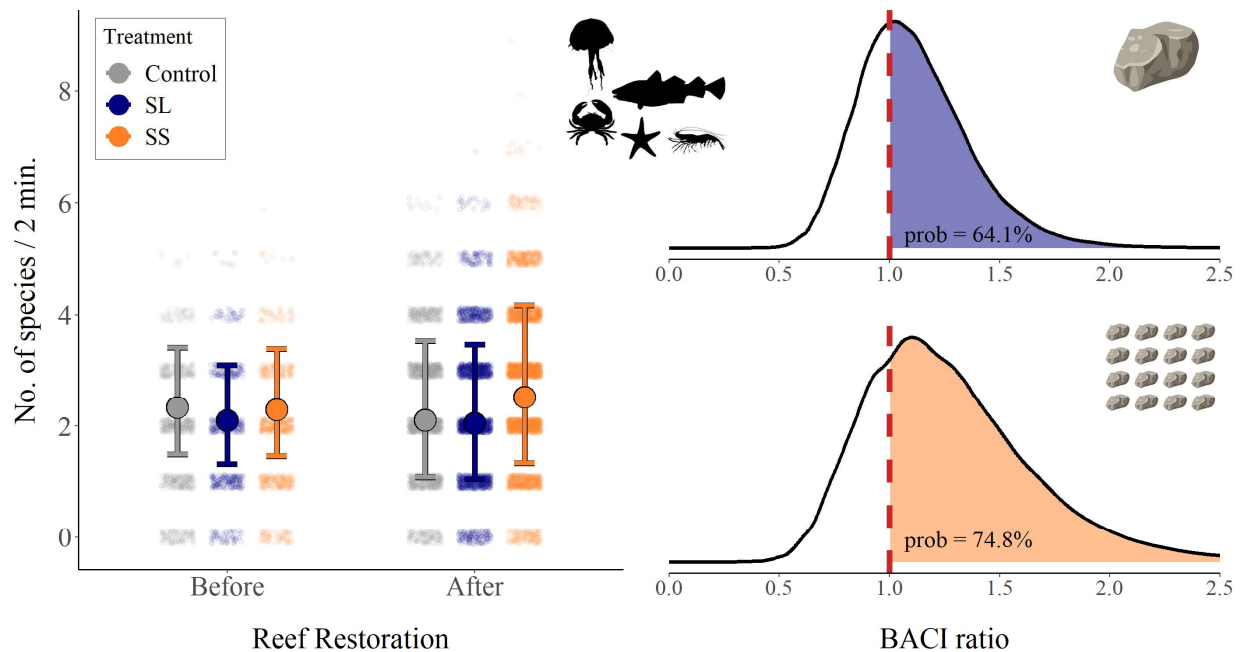
Figure 3.2 – Reef restoration effects on species richness (No. of species / 2min. video). Restoration involved deployment of a single large (SL) reef and several small (SS) reefs. Left-hand side plot shows the posterior means and 95% credible intervals, with small semi-transparent circles representing raw observations that are jittered to improve visual representation. Right-hand side curves show the posterior distribution of BACI ratios for both reef configurations (SL and SS). BACI ratios were computed using equations (1) and (2), with a ratio of 1 indicating equal values before and after the restoration (red dashed line). The probability (i.e. area under the curve) of a positive effect is indicated within the shaded area for each treatment.

3.3.2 Reef restoration effects

Species richness (number of species per hourly recording) increased on average 8.5% and 22% at restored SL and SS reefs respectively, relative to control sites (Table 3.2). Notable observations contributing to post-restoration increases included Atlantic pollock (*Pollachius pollachius*) at SL, two species of sculpin (*Myoxocephalus scorpius* and *Taurulus bubalis*) at SS, and rock gunnel (*Pholis gunnellus*) and saithe at both reef treatments (Table S3.2). However, the posterior distribution of BACI ratios indicated substantial uncertainty around the posterior averages, revealing a moderate 64.1% (SL) and 74.8% (SS) probability of a positive restoration effect on species richness (Figure 3.2). The gadoid family showed a strong positive restoration response (Figure 3.3a). Specifically, gadoid abundance increased 60-fold and 129-fold on average at SL and SS, respectively, relative to control sites. The BACI ratio distributions indicated a probability of 99.8% for both configurations that reef restoration resulted in

Table 3.2 – Summary of average BACI ratios (R_{BACI}; computed according to Conner et al., 2016) with 2.5 and 97.5 percentiles of the ratio distributions in parentheses and probabilities (between 0 and 1) of differential restoration effect sizes across the columns.

		R_{BACI}	$P(R_{\text{BACI}} > 1)$	$P(R_{\text{BACI}} > 2)$	$P(R_{\text{BACI}} > 10)$	$P(R_{\text{BACI}} > 100)$
Species richness	SL	1.09 (0.70 – 1.69)	0.64	0.00	0.00	0.00
	SS	1.22 (0.69 – 2.21)	0.75	0.05	0.00	0.00
Gadoids	SL	60.4 (6.1 – 1510)	0.99	0.99	0.95	0.52
	SS	129.2 (7.2 – 4478)	0.99	0.99	0.96	0.66
Labrids	SL	1.8 (0.4 – 12.6)	0.83	0.57	0.05	0.00
	SS	3.6 (0.8 – 24.1)	0.95	0.81	0.17	0.00
Flatfishes	SL	0.34 (0.09 – 1.23)	0.04	0.01	0.00	0.00
	SS	0.35 (0.10 – 1.07)	0.03	0.00	0.00	0.00
	SL	0.4 (0.1 – 2.7)	0.25	0.05	0.00	0.00

Demersal gobies	SS	8.3 (1.8 – 99.8)	0.99	0.97	0.58	0.02
Sand gobies	SL	0.27 (0.04 – 1.32)	0.05	0.01	0.00	0.00
	SS	1.69 (0.20 – 14.1)	0.67	0.40	0.05	0.00

higher gadoid abundance (Figure 3.3a). The labrid family also responded positively to the reef restoration, with 1.8-fold (SL) and 3.6-fold (SS) average increases in abundance, relative to control sites. Probabilities for a positive restoration effect on labrid abundance were moderate for SL at 82.8%, but high for SS at 95.5% (Figure 3.3b).

In contrast, flatfishes showed a negative response to the restoration efforts. The average BACI ratios were similar between reef treatments, with a ratio of 0.35 indicating a 65% decrease on average in flatfish abundance, relative to control sites (Table 3.2). This decrease was also reflected in very low probabilities of 4.4% (SL) and 3.1% (SS) of a positive restoration effect (Figure 3.3c). Within the goby groups, restoration responses strongly depended on reef configuration. Demersal gobies decreased on average 60% in abundance at SL compared with control sites, with a 24.8% probability of a positive restoration effect (Figure 3.3d). In contrast, demersal gobies increased on average 730% in abundance at SS relative to control sites, with a 99.2% probability of a positive restoration effect. Sand gobies decreased 73% in abundance at SL contrary to a 69% increase at SS, relative to control sites, yielding a 5.1% probability of a positive restoration effect at SL, compared with 66.7% at SS reefs (Figure 3.3e).
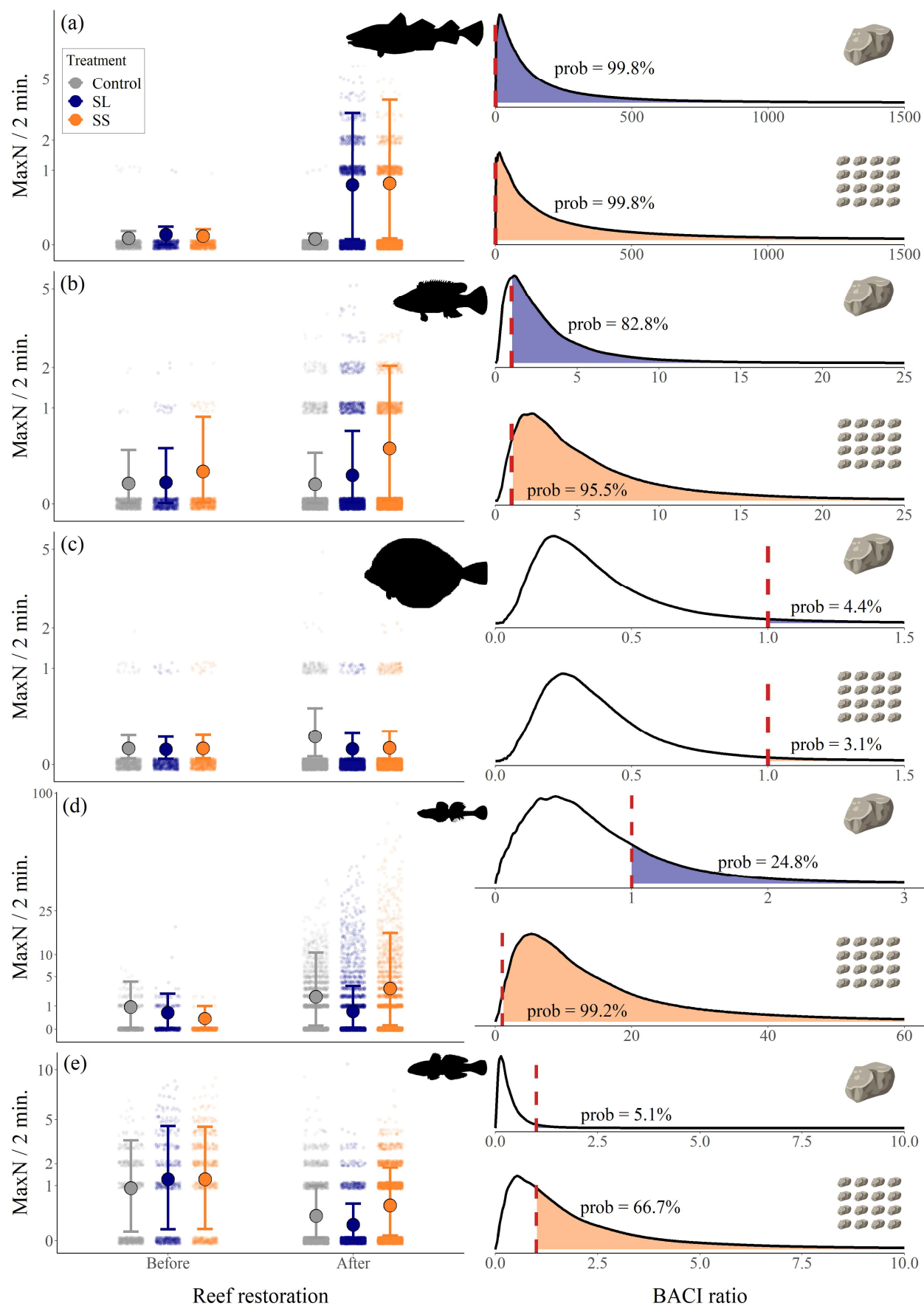
Figure 3.3 – Reef restoration effects on relative abundance (MaxN / 2min. video) of the most prevalent taxa. Interpretation of left- and right-hand side plots is described in caption of Figure 3.2. Y-axes in left-hand side plots were root-transformed to highlight fine-scaled variations in abundances between treatments. Density curves on the right-hand side show the posterior distributions of BACI ratios for all focal taxa, including (a) gadoids (*Gadidae* spp.), (b) labrids (*Labridae* spp.), (c) flatfishes (*Pleuronectiformes* spp.), (d) demersal gobies (*Gobiidae* spp.) and (e) sand gobies (*Gobiidae* spp.).

3.3 Reef configuration effects

Configuration of the reefs had a moderate effect on species richness, with on average 15% more species at SS and a probability of 70.3% that SS promoted richness over SL (Figure 3.4a). For gadoids, we found an average 1.6 times higher abundance at SS compared with SL and a 65.6% probability that SS promoted gadoid abundance (Figure 3.4b). Labrid abundance was on average 1.9 times higher at SS relative to SL with a 78.5% probability of higher labrid abundance at SS (Figure 3.4c). The negative restoration response of flatfish was not affected by the reef configuration, as indicated by the 52.7% probability of higher abundance at SS (Figure 3.4d). The average SLOSS ratio for demersal gobies indicated that 26.7 times more gobies were observed at SS relative to SL reefs, while the density curve showed a 99.6% probability that SS resulted in higher demersal goby abundance (Figure 3.4e). Similarly, sand gobies were on average 7.9 times more abundant at SS, with a 98.6% probability of SS promoting sand gobies relative to SL reefs (Figure 3.4f).
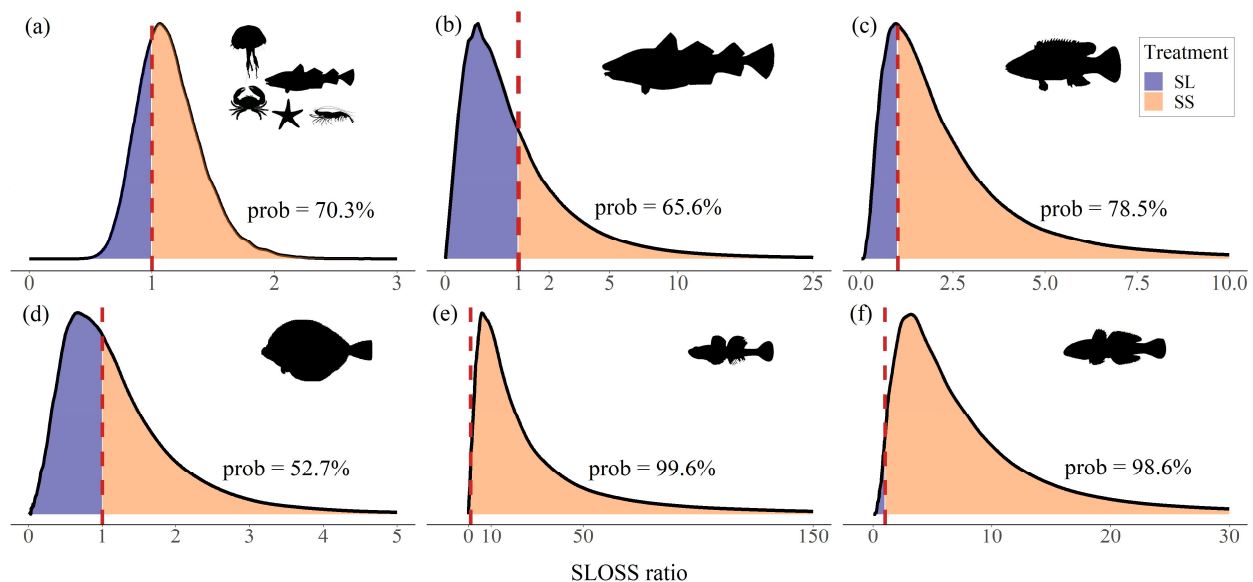
Figure 3.4 – Posterior distributions of SLOSS ratios computed according to equation (3). SLOSS ratios represent a direct comparison of the restoration effect at SL versus SS. A ratio of 1 corresponds to an equal restoration effect for both reef treatments (red dashed line). The probability (area under the curve) of a larger response variable at SS

relative to SL sites is indicated next to each curve. SLOSS posterior distributions are shown for: (a) species richness; (b) gadoids (*Gadidae* spp.); (c) labrids (*Labridae* spp.); (d) flatfishes (*Pleuronectiformes* spp.); (e) demersal gobies (*Gobiidae* spp.) and (f) sand gobies (*Gobiidae* spp.).

3.3.4 Community composition

Results from the LVMs indicated a unique species composition at the restored reefs that was clearly distinct from pre-restoration sites and post-restoration controls (Figure 3.5a). The distinct composition at restored reefs was mainly driven by large-bodied piscivores; including cod (*G. morhua*), saithe (*P. virens*) and greater sandeel (*H. lanceolatus*), and a ctenophore; Northern comb jelly (*B. infundibulum*). Furthermore, the exclusion of reference sites revealed distinct communities at SL and SS reefs (Figure 3.5b), with a number of small-bodied mesopredators (*G. niger*, *Caridea sp.* and *C. maenas*) showing associations with SS. Vegetation coverage was substantially higher at one of the SL sites (Figure S3.3), yet accounting for this environmental factor in a correlated response model indicated that vegetation was not the driver of the distinct communities, as evidenced by the lack of overlap between the confidence ellipses (Figure 3.5c). Instead, accounting for reef configuration resulted in no residual patterns (Figure 3.5d), confirming that SLOSS was the main driver of the distinct communities.

3.4. Discussion

This study restored boulder reefs to recover hard substrate availability for reef-associated fauna in a coastal area of the Baltic Sea, where century-long boulder extraction has severely degraded and removed large areas of reef habitat. We constructed reefs in a SL and SS design to examine the importance of spatial habitat configuration for reef restoration in terms of colonizing marine taxa. Video monitoring revealed that a range of marine species colonized the reefs within six months. Most notably, the strong increases in relative abundance of commercially exploited gadoids suggest that boulder reef restoration is a promising management tool to help recover predatory fish stocks in degraded temperate waters. Spatial reef configuration did not have a pronounced effect on species richness, but instead triggered taxon-specific responses with small-bodied mesopredators strongly promoted at SS. Our results indicate that SLOSS should become an important facet within restoration strategies and highlight the importance of collecting relative abundance data for taxonomic groups to move beyond the traditional focus on species richness and presence-absence data in evaluating SLOSS dynamics (Deane, Nozohourmehrabad, Boyce, & He, 2020).

The combination of MCMC sampling and BACI ratio computation allows for easy to interpret probabilistic inference on the effect of impact events (Conner et al., 2016) and revealed that reef

restoration in this study had a high probability of strongly increasing gadoid abundance (Table 3.2). These results confirm the importance of hard substrate for gadoids as highlighted in previous studies (Gotceitas & Brown, 1993; Rhodes et al., 2020), and stress the need for continued reef conservation and restoration
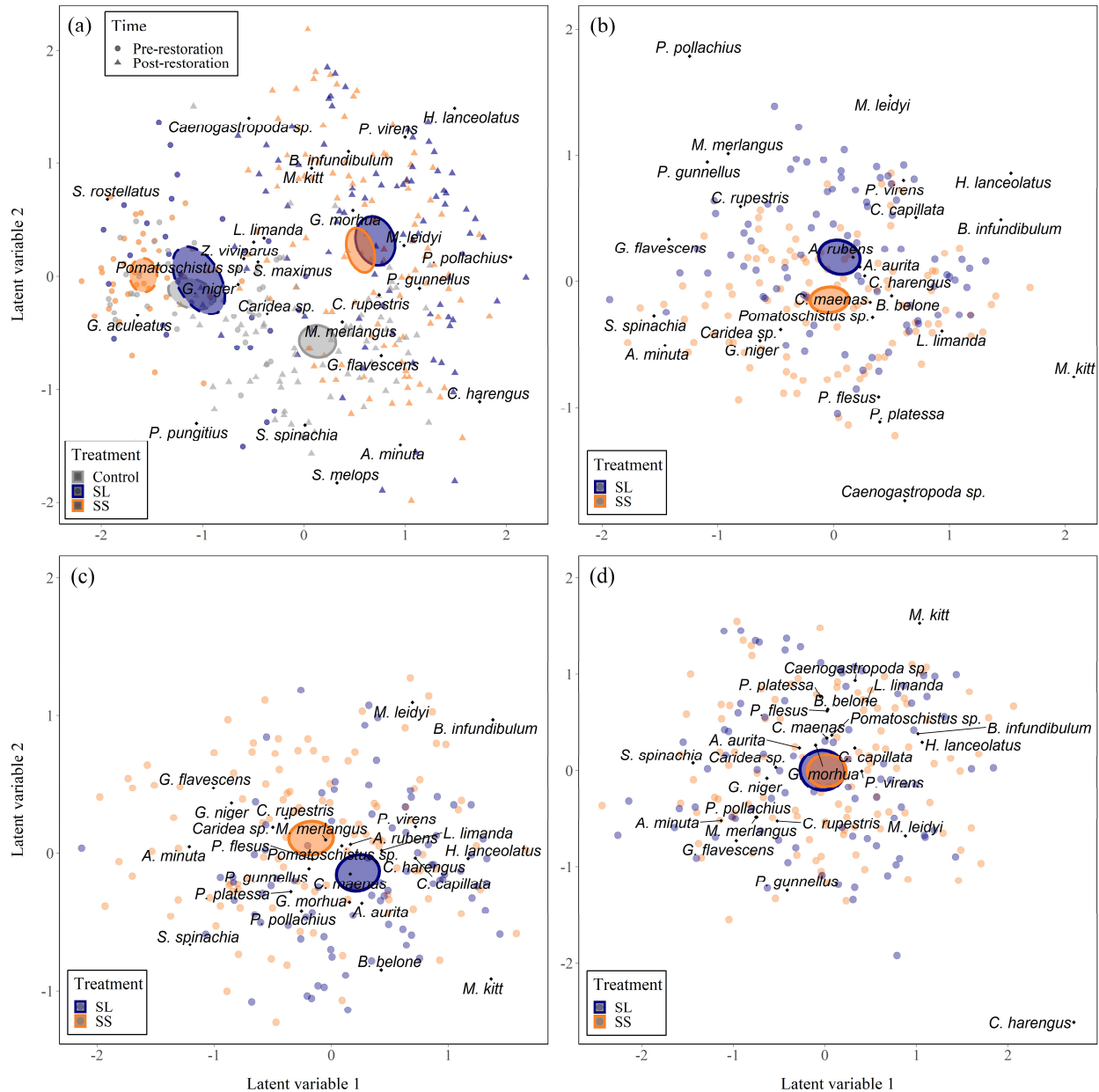
Figure 3.5 – Biplots of unconstrained ordinations based on pure latent variable models (LVMs) and residual ordinations from correlated response models (CRMs). Species composition for each camera deployment is regressed against a set of unknown parameters (the latent variables) for low-dimensional visualization of community patterns. Each of the small symbols corresponds to a single camera deployment, while ellipses represent the 95% confidence intervals for the centroids of pre-restoration (dashed ellipses) and post-restoration (solid ellipses) treatments. LVMs were run on all data pooled to assess restoration effects (a) and separately on post-restoration reef data to assess the SLOSS effects (b) on species composition. To identify the driver(s) of distinct community patterns between

restored SL and SS reefs, we assessed residual ordinations from CRMs that included vegetation coverage (c) and spatial reef configuration (d) as environmental variables. Positions of the 25 most important indicator species (based on their latent variable coefficients) are superimposed as small black dots to visualize their association with the different time-treatment combinations. For example, species showing positive coefficients for both latent variables in (a) are associated with the restored reefs (e.g. *P. virens* and *H. lanceolatus*), whereas a negative latent variable 1 coefficient indicates an association with pre-restoration sites (e.g. *G. aculeatus*).

efforts in areas where gadoids are heavily exploited, such as the Baltic Sea. Labrids and demersal gobies also responded favorably to the reef restoration, as expected from their strong association with diverse reef structures such as holes and crevices (Sayer, Gibson, & Atkinson, 1993; Utne-Palm, Eduard, Jensen, Mayer, & Jakobsen, 2015). As common mesopredators, labrids and gobies may exert strong top-down effects on macrophyte communities by reducing mobile mesograzers (Östman et al., 2016). Management actions promoting healthy piscivorous fish stocks can therefore strengthen top-down controls and prevent cascading negative effects from mesopredator release (Östman et al., 2016). While restoration efforts seemed to trigger an increase in mesopredators in this study, the concurrent increase in gadoids suggests that reef restoration may reinforce top-down controls by piscivorous fishes and possibly enhance resilience in degraded marine systems.

In contrast, flatfishes responded negatively to the reef restoration independent of the configuration of the reefs. Since flatfishes are ambush predators found predominantly on featureless sandy bottoms (Vinagre, França, Costa, & Cabral, 2005), the decrease in flatfish abundance in our study is a direct effect of the decline in soft-bottom area utilized by flatfish at the site-level (~3250 m^2). However, similar to offshore wind farms (Glarou, Zrust, & Svendsen, 2020), adverse effects at such small spatial scales are unlikely to impact population levels of soft-bottom species, particularly in regions with extensive soft-bottom areas such as the South Baltic Sea (Kaskela & Kotilainen, 2017). In general, we note that our results are merely representative of the early stages of succession following the reef restoration. At time of sampling, the six-month old reefs were overgrown with ephemeral algae which support low faunal density and richness (Christie, Norderhaug, & Fredriksen, 2009), implying that the associated community may change over time if reefs are gradually colonized by macroalgae of higher complexity and longevity. Consequently, a well-developed reef could indirectly promote soft-bottom species (e.g. flatfish) in the reef vicinity, by inducing spillover effects on infaunal prey communities from algal food sources (Posey & Ambrose, 1994). However, the reef construction could also adversely affect ecosystem resilience over time by facilitating the colonization of invasive species, with potential deleterious effects on native taxa (Bulleri & Airoldi, 2005). Two invasive species in the Baltic that are sufficiently conspicuous for RUVS sampling include the round goby (*Neogobius melanostomus*) and comb jelly (*Mnemiopsis leidyi*). We did not record any round gobies in this study and found no evidence that the restored reefs promoted comb jelly, which is a holoplanktonic species (Jaspers, Marty, & Kiørboe, 2018), relative to reference sites (Table S3.2). Still, these mechanisms highlight the importance of

investigating effects of reef construction on soft-bottom taxa (Puckeridge et al., 2021) and of conducting long-term monitoring studies to assess temporal variabilities in restoration effects.

Our study is the first to link the evaluation of reef restoration with SLOSS by examining if restoration efforts can be optimized by manipulating the spatial configuration of a given volume of reef material. We found no pronounced SLOSS effect on richness of early colonizers, yet there were strong taxon-specific differences in relative abundance among the focal groups. There is general agreement within the SLOSS literature that contiguous habitats are vital in supporting large and stable population sizes, partly explained by higher niche and resource availability relative to small patches (Root, 1973). However, there is still a limited understanding of how habitat fragmentation drives relative abundances among taxa, which ultimately depends on functional traits (e.g. body size and feeding strategy), species interactions (e.g. predator-prey dynamics) and the surrounding environment (e.g. matrix habitat and connectivity; Deane et al., 2020; Drakare, Lennon, & Hillebrand, 2006; Ewers & Didham, 2006). The sites in our study represent benign habitat matrices (sensu Fahrig, 2020), in which the aquatic medium allows organisms to cross habitat boundaries from reef to sand patches and vice versa, as opposed to a hostile matrix (e.g. water for insular mammals) with limited permeability for inhabitant species. We note that effects of spatial reef configuration were most apparent in gobies and to a lesser degree in labrids (Figure 3.4), and suggest that the observed SLOSS patterns likely resulted from a combination of high intra- and inter-site connectivity with trait-mediated responses across focal taxa. First, gobies and labrids are small-bodied generalists, feeding on a variety of mesograzers and benthic invertebrates (Fjøsne & Gjøsæter, 1996; Miller, 1986). A small body size implies lower area requirements to establish high population densities (Marquet, Navarrete, & Castilla, 1990), while a generalist feeding style facilitates rapid colonization and efficient resource exploitation within the habitat matrix (Holt, 2014). Therefore, SS may have provided sufficient area for high densities of small-bodied taxa at the patch-level (i.e. single reef patch in Figure 3.1c) and richer food sources at the site-level through a higher perimeter-area ratio (i.e. more edge habitat) yielding increased access to infaunal prey, relative to SL. Second, gobies and labrids are sedentary reef fishes relative to transient gadoids. While sedentary species are highly associated with fine-scaled habitat within the matrix, transient species respond to habitat features on seascape scales (i.e. km's) with species-specific drivers determining visitation rates to reef patches (Harborne, Selwyn, Lawson, & Gallo, 2017). Our results are reflective of this pattern, as the difference in fine-scaled habitat features between SL and SS (e.g. amount of edge habitat or relative reef isolation) did not affect the abundance of transient gadoids at the site-level. Still, the spatial distribution of gadoids was clearly more dispersed at SS as opposed to spatially aggregated at SL (Figure S3.12), which likely caused increased mortality of prey species at SL (Overholtzer-McLeod, 2006) and thereby SS > SL for small-bodied taxa at the site-level. Interestingly, the higher abundance of sand gobies at SS suggests that this configuration could benefit soft-bottom species in addition to reef species. Similar to small-bodied reef taxa, we hypothesize that this was partly a predator-mediated effect since the spatial distribution of sand gobies at SL was seemingly inversely related to the distribution of gadoids (Figure S3.12; S3.13). We note that

effects on soft-bottom species would remain obscured when merely sampling focal habitat (here reef patches) and therefore argue that studies on habitat fragmentation within highly connected systems require a sampling design incorporating the entire habitat matrix to assess community dynamics across habitat boundaries.

Finally, the aquatic medium facilitates immigration and emigration of organisms among local populations and enables marine taxa with sedentary adult life stages to colonize and replenish relatively isolated habitats via larval dispersal in the water column (Carr et al., 2003). This implies that the observed SS > SL pattern in sedentary taxa could have resulted from differential larval input if the SS configuration yielded higher larval settlement and survival, relative to SL reefs. Investigating the effect of spatial reserve design on oyster recruitment, Puckett & Eggleston (2016) found that SS reserves promoted greater larval connectivity than a few large reserves, which mostly relied on local larval retention. For reef fishes, there is increasing evidence that local larval retention (or self-recruitment) is more common than previously considered (Almany et al., 2017; Jones et al., 2009). While we were unable to infer the relative contribution of larval dispersal and retention at the two reef designs, we hypothesize that both mechanisms could result in lower larval settlement and survival at SL, relative to SS. At the patch-level, distance to the nearest reef patch was 15 m at SS (Figure 3.1c), but 500-1500 m for SL (distance between sites). At the site-level, the higher perimeter-area ratio at SS could increase the rate at which larvae encounter or locate the reef habitat (McNeill & Fairweather, 1993). Therefore, the combined effects of relative isolation, lower perimeter-area ratio and aggregation of predators at SL may have substantially reduced larval settlement and survival, relative to SS. We note however, that distance to the nearest reef for SL (500-1500 m) is still within range even of short-distance dispersers (1.7 km median distance for a confamilial goby; D'Aloia et al., 2015) and that larval connectivity is more likely shaped by geographic setting rather than the fine-scaled habitat features of SL and SS in our study (Jones et al., 2009). As such, there is a promising opportunity in emerging technologies such as otolith microchemistry to provide insights into source populations of larvae and the effect of habitat boundaries on dispersal capabilities of aquatic organisms (Kaemingk, Swearer, Bury, & Shima, 2019; Rogers, Fowler, Steer, & Gillanders, 2019).

In conclusion, we demonstrated that boulder reef restoration is a promising tool to recover essential habitat for reef-associated fauna in degraded aquatic systems. The positive restoration effects on gadoids are particularly noteworthy and highlight the importance of continued reef conservation and restoration efforts to help sustain commercially exploited gadoid populations in the Baltic Sea. We acknowledge that large individuals recorded in our study were most likely attracted to the newly constructed reefs instead of locally produced (alluding to the 'attraction versus production' debate; Bohnsack, 1989), since the reefs were only six months old at time of sampling. However, we concurrently note that we selected restoration sites based on historical knowledge and archived nautical maps to target sites where reefs were historically present but extracted entirely over the past decades. As such, this fundamental shift in reef

availability likely rendered hard substrate to become a limiting factor for reef-associated fauna at the study area, implying substantial scope for productivity enhancement from reef construction over time (Folpp et al., 2020; Layman & Allgeier, 2020). We furthermore acknowledge that our results are based on a small number of reef replicates (two replicates per treatment) and that the study area of the Baltic Sea is characterized by unique conditions of low salinity levels and a multitude of anthropogenic stressors (Reusch et al., 2018). However, we emphasize that our results should be interpreted strictly within a substrate-limiting context and accordingly suggest that boulder reef restoration likely constitutes a similarly promising management action to help recover reef-associated fish populations in other temperate waters where hard substrate is limiting. SLOSS remains an important issue for conservation biology, as managers and practitioners often prioritize the preservation of contiguous, large habitats over several small patches, despite the majority of empirical evidence highlighting the conservation value of small habitats (Fahrig, 2020). From a restoration ecology perspective, we showed that restored SS reefs promoted early colonization of small-bodied mesopredators while none of the response variables were elevated at SL relative to SS reefs. Still, we note that this does not necessarily imply a higher restoration success rate at SS. Specifically, large numbers of mesopredators could exert cascading top-down effects in favor of ephemeral algae (Östman et al., 2016), potentially hampering the development of macrophyte communities with higher complexity and longevity at SS over time. While both SL and SS strongly promoted predatory gadoids of higher trophic level, suggesting a high potential for top-down control on mesopredators, the true extent of trophic resilience at the restored reefs could not be assessed from the fish community metrics alone. This highlights a need for future restoration efforts to incorporate trophic dynamics within the BACI framework to obtain a more comprehensive evaluation of restoration success (Loch, Walters, & Cook, 2020).

Authors' contributions

JCS, JGS, HB and BMK designed the study; TW, PN, JCS and BMK collected the data; TW, PN and HB analyzed the data; TW led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Conflict of Interest

The authors declare no conflicts of interest with the publication of this article.

Acknowledgements

Data availability statement

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.vt4b8gtq8 (Wilms et al., 2021a). An R script with computational details for running the analysis and produce the figures is archived on Zenodo https://doi.org/10.5281/zenodo.5268843 (Wilms et al., 2021b).

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Supporting information

S3.1 – Sampling procedures and RUVS positioning

Field sampling depended strongly on wind force and direction, with winds below 6-7 m/s deemed suitable for boating to the study area (Fig. 3.1). Throughout periods of suitable conditions, field sampling was carried out on a daily basis with cameras being re-deployed after a minimum of 24 hours. Accordingly, we conducted a total of 12 sampling days in 2016 (pre-restoration) and 25 sampling days in 2018 (post-restoration). On windy days, cameras were left recording in the field until either the battery life ran out or weather conditions improved and allowed for re-deploying. This resulted in 24-60 hourly recordings per camera deployment, which included recordings at night that were later discarded as no artificial lighting was used in this study. We subsequently accounted for this variation in hourly recordings by defining group-level effects per Deployment ID nested within Site ID to estimate varying intercepts for each camera deployment. Apart from incorporating dependencies within the grouping levels (i.e. hourly recordings from the same camera are not completely independent), this hierarchical model structure allows for unequal sample sizes between groups by sharing information across grouping levels (i.e. partial pooling) to estimate the population of varying intercepts in the lowest model hierarchy (Nalborczyk, Batailler, Loevenbruck, Vilain, & Bürkner, 2019).

We deployed a maximum of 3-4 remote underwater video systems (RUVS) at the same field site on a given sampling day, while maximizing their distance within confines of the sites. Field sites were divided into three zones according to the distance from the site's center (A, 0-11m; B, 11-22m; C, 22-33m from the center; Fig. S3.1) and RUVS were randomly assigned a position and direction of view within each of the zones. This scheme was adopted to ensure equal sampling coverage of the matrix (i.e. site) interior, intermediate and outer region while randomizing the intra-zone positioning of the RUVS. For example, if one RUVS was deployed in north-east direction within zone A, a second RUVS would be deployed at a random location in south-west direction within zone B to maximize the distance between the two RUVS.
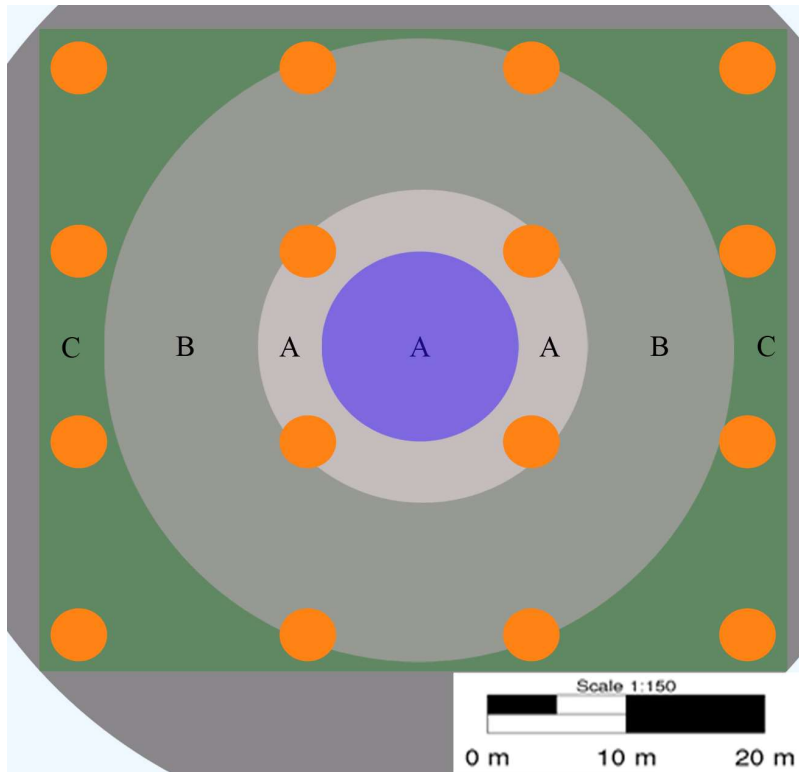
Figure S3.1 – Schematic drawing of the sampling procedure. The green square outlines the confines of the field site within which video samples were collected. The position of the single large (SL) reef is indicated with the large blue circle, while several small (SS) reefs are represented by the small orange circles. Note that while SL and SS are presented together in this schematic drawing for visual purposes, the two reef treatments were constructed across separate replicate field sites in our study (Fig. 3.1). All field sites were divided into 3 zones, A (0-11 m from center); B (11-22 m from center) and C (22-33 m from center).

The spatial arrangement at SS sites allowed us to optimize our sampling effort to four RUVS deployments at a given sampling day, with the fourth RUVS randomized between the zones. However, we were unable to mimic this strategy at SL sites, due to complex reef structures (i.e. holes and crevices) preventing deployment of more than one RUVS within zone A (i.e. the SL reef top). RUVS within zone A at SL sites would occasionally slide between the reef structures, which required a substantial radius around the RUVS free of other deployments in order to retrieve the camera system from the boat. Therefore, a maximum of three RUVS were run at a time at SL sites (one within each zone) resulting in a variation in the sampling effort between SL and SS (Table 3.1). However, proportions of samples at each

habitat type (i.e. reef and sand habitat) were roughly equal between the reef treatments, with about one-third of the deployments at SL and SS recording reef habitat and two-thirds landing on sand habitat (Table S3.1). In addition, we defined varying intercepts per Site ID in the models, similar to varying intercepts per Deployment ID described above (but one level higher up the model hierarchy), to account for the variation in sample sizes between sites (Table 3.1).

Table S3.1 – Proportions of post-restoration RUVS deployments that landed either on sand or reef habitat. Proportions are given for the single large (SL) and several small (SS) reef treatments, as well as for the replicate field sites.

	SL		SS	
Sand habitat	0.686		0.661	
Reef habitat	0.314		0.339	
	SL1	SL2	SS1	SS2
Sand habitat	0.682	0.690	0.673	0.649
Reef habitat	0.318	0.310	0.327	0.351
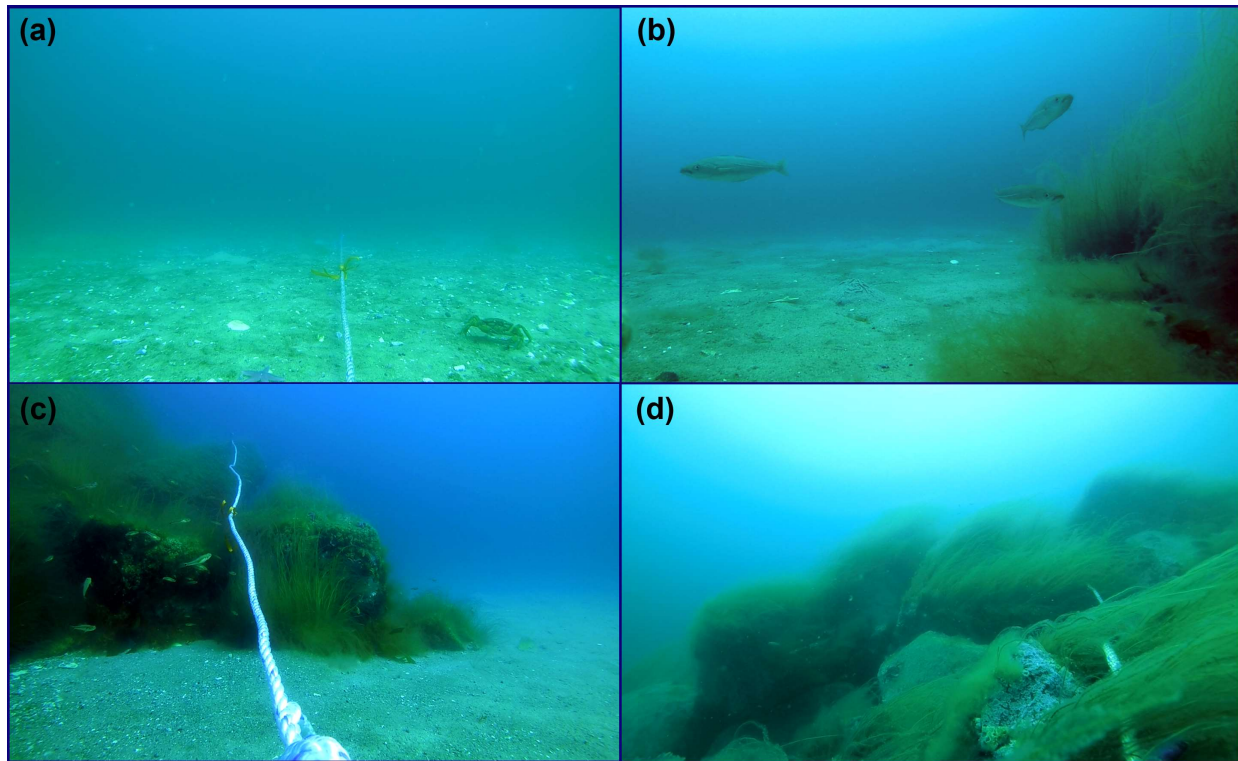
Figure S3.2 – Examples illustrating varying boulder coverage (BC) and vegetation coverage (VC) from RUVS video clips. Video observers estimated BC and VC as proportions of the visible seabed, to describe the sampled habitat. (a) Recordings of sandy bottom were assigned an equal BC and VC value of 0.00, here shown together with a common shore crab (*C. maenas*). (b) Reef edge with mostly sandy bottom visible and all boulders covered with vegetation (here BC = VC = 0.25), also shown with three individuals of saithe (*P. virens*). (c) Reef edge with higher proportion of seabed covered with boulders relative to (b) (BC = 0.40), but boulders not entirely overgrown with vegetation (VC = 0.25), also showing a school of two-spotted gobies (*G. flavescens*). (d) Reef top recording with no visible sandy bottom (BC = 1.00 or 100%) and boulders not entirely overgrown with vegetation (VC = 0.75 or 75%).

Table S2 – Complete list of species observations from the video recordings. Observations are grouped per time level (i.e. before and after the restoration) and treatment level (i.e. Control, SL and SS). The values for each observation indicate the frequency of occurrence, i.e. the percentage (0-100%) of samples (two-minute recordings) for which the given species was observed. The total number of samples (N) is provided in the column headers for each treatment level before and after the reef restoration. Values in brackets correspond to the mean MaxN (i.e. maximum number of individuals within a video frame) for those samples in which the given species was observed. Species names marked with symbols indicate the focal species analyzed within the five most prevalent taxa in this study: * Gadoids (*Gadidae spp.*); † Labrids (*Labridae spp.*); ‡ Flatfishes (*Pleuronectiformes spp.*); § Demersal gobies (*Gobiidae spp.*); ¶ Sand gobies (*Gobiidae spp.*).

Species observed			Pre-restoration			Post-restoration		
Family / Order	Scientific name	Common name	Control (N=870)	SL (N=585)	SS (N=732)	Control (N=2148)	SL (N=2306)	SS (N=3308)
Agonidae	Agonus cataphractus	Armed bullhead	0 (0)	0 (0)	0 (0)	0.05 (1)	0 (0)	0.03 (1)
Ammodytidae	Hyperoplus lanceolatus	Greater sandeel	0 (0)	0 (0)	0 (0)	0.8 (10.3)	1.3 (12.3)	1.8 (6.9)
	Ammodytidae sp.	Unidentified sandeel	0 (0)	0 (0)	0 (0)	0.2 (24.5)	0.3 (1.17)	0.4 (1.25)
Asteriidae	Asterias rubens	Common starfish	12.8 (1.12)	17.6 (1.31)	16.8 (1.48)	5.4 (1.16)	30.6 (1.79)	40.5 (2.03)
Belonidae	Belone belone	Garfish	0.12 (2)	0.34 (1)	0 (0)	0.56 (1)	0.52 (1)	0.18 (1)
Bolinopsidae	Bolinopsis infundibulum	Northern comb jelly	0 (0)	0 (0)	0 (0)	0.05 (1)	0 (0)	0.12 (1)
	Mnemiopsis leidyi	Warty comb jelly	0 (0)	0 (0)	0 (0)	0.6 (1)	0.8 (1.03)	0.2 (1)
Clupeidae	Clupea harengus	Atlantic herring	0.12 (1)	0 (0)	0 (0)	0.56 (36.5)	0.09 (56.5)	0.42 (34.6)
	Clupeidae sp.	Unidentified clupid	0 (0)	0 (0)	0 (0)	0.05 (28)	0.04 (84)	0 (0)
Cottidae	Myoxocephalus scorpius	Shorthorn sculpin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.03 (1)
	Taurulus bubalis	Longspined bullhead	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.03 (1)
	Cottidae spp.	Unidentified sculpin	0 (0)	0 (0)	1.50 (1)	0.09 (1)	0.04 (1)	0.09 (1)
Crangonidae	Crangon crangon	Common shrimp	0 (0)	0.17 (1)	0.27 (1)	1.91 (1.12)	0.22 (1)	0.63 (1)
Cyaneidae	Cyanea capillata	Lion's mane jellyfish	7.13 (1.08)	4.79 (1.14)	3.83 (1)	34.9 (1.86)	27.5 (1.81)	19.0 (1.72)
Gadidae	Gadus morhua*	Atlantic cod*	0.23 (1)	0.34 (1)	0 (0)	0.19 (1)	19.4 (1.37)	11.7 (1.18)
	Pollachius virens*	Saithe*	0 (0)	0 (0)	0 (0)	0 (0)	9.76 (1.31)	6.11 (1.36)
	Pollachius pollachius*	Atlantic pollock*	0 (0)	0 (0)	0 (0)	0 (0)	0.69 (1.13)	0 (0)
	Merlangius merlangus*	Whiting*	0.23 (1)	0 (0)	0 (0)	0.05 (1)	0.17 (1)	0.06 (1)
	Gadidae spp.*	Unidentified gadoid*	0.46 (1)	0 (0)	0.14 (1)	0.09 (1)	5.68 (1.22)	3.14 (1.14)
Gasterosteidae	Gasterosteus aculeatus	Three-spined stickleback	0.34 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Pungitius pungitius	Nine-spined stickleback	0.23 (1.5)	0.51 (1)	0.27 (1)	0 (0)	0 (0)	0 (0)
	Spinachia spinachia	Fifteen-spined stickleback	0 (0)	0 (0)	0 (0)	1.16 (1)	0.22 (1)	0 (0)
Gobiidae	Aphia minuta §	Transparent goby §	0 (0)	0.9 (5.2)	0 (0)	13.0 (4.14)	2.2 (3.41)	4.4 (3.75)
	Gobius niger ¶	Black goby ¶	3.6 (1.03)	2.7 (1.25)	6.4 (1.02)	0.5 (1)	0 (0)	1.8 (1)
	Gobiusculus flavescens §	Two-spotted goby §	17.2 (1.72)	11.8 (1.39)	1.8 (1.38)	55.6 (3.29)	39.2 (5.90)	40.5 (6.54)
	Pomatoschistus sp. ¶	Sand goby ¶	40.9 (1.87)	50.9 (2.34)	64.8 (2.41)	26.0 (1.63)	12.4 (1.37)	45.6 (1.49)
Labridae	Ctenolabrus rupestris †	Goldsinny wrasse †	0.92 (1.25)	3.42 (1.1)	2.60 (1.11)	11.6 (1.2)	19.2 (1.33)	17.5 (1.21)

Family	Species	Common name						
	Symphodus melops †	Corkwing wrasse †	0 (0)	0.34 (1)	0 (0)	0 (0)	0.04 (1)	0.18 (1)
Paguridae	*Pagurus bernhardus*	Soldier crab	0.46 (1)	0 (0)	0.14 (1)	0 (0)	0 (0)	0 (0)
Phocoenidae	*Phocoena phocoena*	Harbour porpoise	0 (0)	0 (0)	0 (0)	0.05 (1)	0 (0)	0.03 (1)
Pholidae	*Pholis gunnellus*	Rock gunnel	0 (0)	0 (0)	0 (0)	0 (0)	0.13 (1)	0.21 (1)
Pleuronectidae	*Limanda limanda* ‡	Common dab ‡	0.81 (1)	0.86 (1)	0.41 (1)	0.65 (1.07)	0.13 (1)	0.73 (1.04)
	Platichthys flesus ‡	European flounder ‡	0.23 (1)	0 (0)	0.14 (1)	0.70 (1)	0.04 (1)	0.36 (1)
	Pleuronectes platessa ‡	European plaice ‡	0.23 (1)	0 (0)	0.27 (1)	0.23 (1)	0.13 (1)	0.15 (1)
	Microstomus kitt ‡	Lemon sole ‡	0 (0)	0 (0)	0 (0)	0.14 (1)	0 (0)	0.09 (1)
	Pleuronectidae spp. ‡	Unidentified flatfish ‡	0.57 (1.2)	0.51 (1)	1.23 (1)	2.61 (1.04)	0.87 (1)	1.24 (1.02)
Portunidae	*Carcinus maenas*	Shore crab	27.1 (1.18)	26.7 (1.19)	25.4 (1.15)	35.5 (1.26)	31.0 (1.43)	55.2 (1.75)
	Liocarcinus pusillus	Dwarf swimming crab	0 (0)	0.17 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Scophthalmidae	*Scophthalmus maximus* ‡	Turbot ‡	0 (0)	0.17 (1)	0 (0)	0.09 (1)	0 (0)	0.06 (1)
	Scophthalmus rhombus ‡	Brill ‡	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.03 (1)
Soleidae	*Solea solea*	Common sole	0 (0)	0.17 (1)	0.14 (1)	0 (0)	0 (0)	0 (0)
Syngnathidae	*Syngnathus rostellatus*	Nilsson's pipefish	0.92 (1.25)	0 (0)	0.14 (1)	0 (0)	0 (0)	0 (0)
	Syngnathus typhle	Broadnosed pipefish	0 (0)	0 (0)	0.14 (1)	0 (0)	0 (0)	0 (0)
Ulmaridae	*Aurelia aurita*	Moon jelly	52.9 (1.89)	50.8 (1.84)	49.3 (1.73)	31.1 (1.72)	22.2 (1.54)	17.0 (1.48)
Zoarcidae	*Zoarces viviparus*	Common eelpout	0.12 (1)	0 (0)	0.14 (1)	0 (0)	0 (0)	0.06 (1)
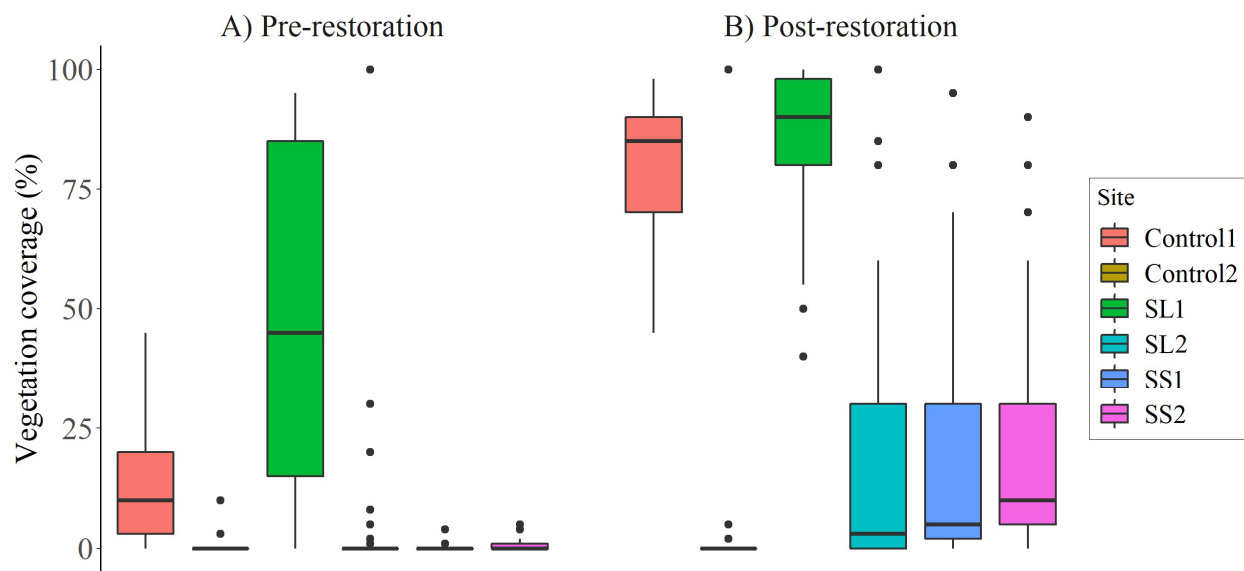
Figure S3.3 – Vegetation coverage across the field sites estimated from recordings before (A) and after (B) the reef restoration. While the hard substrate provided by the restored rocky reefs promoted the anchorage and growth of vegetation (i.e. macroalgae) at restoration sites (SL and SS), it is noteworthy that vegetation coverage also increased at one of the control (i.e. soft-bottom) sites. Vegetation at the control site was observed to be attached to small hard substrates (e.g. pebbles or shells). This trend illustrates the presence of inherent natural fluctuations within marine systems and highlights the need for robust sampling designs (e.g. a BACI design) and environmental covariates in the models to disentangle such fluctuations from the impact (restoration) effect of interest.

S3.3 – Model summaries

Table S3.3 – Model description of the most parsimonious GLMMs for each of the six focal response variables. Since all response variables are integers, all distributions used belong to the exponential family, including the Poisson, Conway-Maxwell Poisson (CMP), negative binomial with variance increasing quadratically with the mean (NB2) and the zero-inflated negative binomial (ZINB). Priors are listed based on prior predictive checks according to Gabry et al. (2019; example provided in Fig. S3.1). Correlation structures used include a Gaussian process (GP; exponentiated-quadratic kernel) and an autoregressive (AR) structure in case spatial and/or temporal dependencies were present respectively. Priors on the GP length scale parameter (ρ) were set using the STAN algebraic solver.

Response variable	Distribution	Priors	Correlation structure	Post warm-up iterations
Species richness	CMP	$\beta_0 \sim N(0,2)$ $\beta_{1-6} \sim N(0,2)$ $\sigma_{site} \sim hN(0,1)$ $\rho_{lon_{before}} \sim InvGamma(0.778, 0.002)$ $\rho_{lat_{before}} \sim InvGamma(0.829, 0.002)$ $\rho_{lon_{after}} \sim InvGamma(0.781, 0.002)$ $\rho_{lat_{after}} \sim InvGamma(0.831, 0.002)$ $\alpha_{marg} \sim hN(0,3)$ $\rho_{AR} \sim N(0,1)$ $\sigma_{err} \sim hN(0,1)$ $v \sim hCauchy(0, 2.5)$	GP + AR	40,000
Gadoid MaxN	NB2	$\beta_0 \sim N(0,3)$ $\beta_{1-6} \sim N(0,3)$ $\sigma_{site}, \sigma_{depl} \sim hN(0,1)$ $\rho_{lon_{before}} \sim InvGamma(0.778, 0.002)$ $\rho_{lat_{before}} \sim InvGamma(0.829, 0.002)$ $\rho_{lon_{after}} \sim InvGamma(0.781, 0.002)$ $\rho_{lat_{after}} \sim InvGamma(0.831, 0.002)$ $\alpha_{marg} \sim hN(0,2)$ $\rho_{AR} \sim N(0,1)$ $\sigma_{err} \sim hN(0,1)$ $\phi \sim Gamma(0.01, 0.01)$	GP + AR	30,000
Labrid MaxN	CMP	$\beta_0 \sim N(0,3)$ $\beta_{1-7} \sim N(0,3)$ $\sigma_{site}, \sigma_{depl} \sim hN(0,1)$ $v \sim hCauchy(0, 2.5)$	-	40,000
Flatfish MaxN	Poisson	$\beta_0 \sim N(0,2)$ $\beta_{1-7} \sim N(0,2)$ $\sigma_{site}, \sigma_{depl} \sim hN(0,1)$ $\rho_{lon_{before}} \sim InvGamma(0.778, 0.002)$ $\rho_{lat_{before}} \sim InvGamma(0.829, 0.002)$	GP	50,000

		$\rho_{lon_{after}} \sim InvGamma(0.781, 0.002)$		
		$\rho_{lat_{after}} \sim InvGamma(0.831, 0.002)$		
		$\alpha_{marg} \sim hN(0,3)$		
Demersal goby MaxN	ZINB	$\beta_0 \sim N(0,3)$ $\beta_{0(zi)} \sim Logistic(0,1)$ $\beta_{1-7} \sim N(0,3)$ $\beta_{1-7(zi)} \sim Logistic(0,1)$ $\sigma_{site}, \sigma_{depl} \sim hN(0,1)$ $\sigma_{(zi)}, \sigma_{site(zi)}, \sigma_{depl(zi)} \sim hN(0,1)$ $\rho_{lon_{before}} \sim InvGamma(0.778, 0.002)$ $\rho_{lat_{before}} \sim InvGamma(0.829, 0.002)$ $\rho_{lon_{after}} \sim InvGamma(0.781, 0.002)$ $\rho_{lat_{after}} \sim InvGamma(0.831, 0.002)$ $\alpha_{marg} \sim hN(0,3)$ $\phi \sim Gamma(0.01, 0.01)$	GP	60,000
Sand goby MaxN	CMP	$\beta_0 \sim N(0,2)$ $\beta_{1-7} \sim N(0,2)$ $\sigma_{site}, \sigma_{depl} \sim hN(0,1)$ $\rho_{AR} \sim N(0,1)$ $\sigma_{err} \sim hN(0,1)$ $v \sim hCauchy(0, 2.5)$	AR	30,000

Table S3.4 – Parameter estimates and diagnostics of the population-level effects for the most parsimonious model for each response variable. The time-treatment interaction (i.e. Year18 x SL and Year18 x SS) is of particular interest within the BACI design, as it corresponds to the difference between control and impact sites across the two sampling years (before and after the reef restoration). The effective sample sizes (ESS) and potential scale reduction ($\hat{R}$) provide model diagnostics, with an $\hat{R}$-value equal to one indicating Markov chain convergence.

Response	Parameter	Estimate	Est. Error	L-95% CI	U-95% CI	Bulk ESS	Tail ESS	R-hat
Species richness	Intercept	0.56	0.09	0.37	0.74	13,815	10,791	1.00
	Year2018	-0.10	0.05	-0.20	-0.01	13,278	16,463	1.00
	SL	-0.09	0.13	-0.35	0.17	13,975	11,561	1.00
	SS	-0.01	0.13	-0.27	0.25	13,429	11,449	1.00
	logVis	0.63	0.02	0.59	0.67	34,274	28,289	1.00
	Year18:SL	0.07	0.07	-0.07	0.21	14,555	21,607	1.00
	Year18:SS	0.17	0.07	0.03	0.30	14,134	20,546	1.00
Gadoid MaxN	Intercept	-6.78	1.19	-9.48	-4.80	10,139	12,569	1.00
	Year2018	0.06	1.04	-1.58	2.54	8,665	8,633	1.00
	TreatSL	0.21	1.40	-2.46	3.16	13,336	14,589	1.00
	TreatSS	-0.30	1.46	-3.05	2.78	13,868	15,000	1.00
	logVis	0.09	0.10	-0.10	0.28	31,476	24,306	1.00
	Year18:TreatSL	4.66	1.17	2.15	6.85	9,595	7,594	1.00
	Year18:TreatSS	5.25	1.23	2.66	7.61	10,837	9,089	1.00
Labrid MaxN	Intercept	-4.05	0.63	-5.33	-2.83	22,895	23,008	1.00
	Year2018	-0.04	0.40	-0.80	0.76	16,144	22,772	1.00
	TreatSL	0.01	0.80	-1.58	1.62	22,039	23,574	1.00
	TreatSS	0.65	0.79	-0.93	2.26	23,710	23,804	1.00
	logVis	0.61	0.09	0.43	0.80	58,770	31,051	1.00
	Vegetation	2.65	0.31	2.07	3.28	10,412	17,913	1.00
	Year18:TreatSL	0.47	0.50	-0.51	1.44	15,396	23,554	1.00
	Year18:TreatSS	0.86	0.49	-0.10	1.83	16,340	23,063	1.00
Flatfish MaxN	Intercept	-4.13	0.39	-4.89	-3.33	22,522	13,367	1.00
	Year2018	0.98	0.46	0.07	1.88	13,855	10,862	1.00
	TreatSL	-0.12	0.54	-1.22	0.94	25,494	24,464	1.00
	TreatSS	0.02	0.51	-1.02	1.04	20,026	10,277	1.00
	logVis	1.09	0.23	0.66	1.54	52,848	37,748	1.00
	Vegetation	-1.58	0.40	-2.40	-0.84	11,155	26,081	1.00
	Year18:TreatSL	-1.13	0.65	-2.38	0.20	19,250	21,933	1.00
	Year18:TreatSS	-1.11	0.59	-2.30	0.07	15,831	11,416	1.00
Demersal goby MaxN	Intercept	-1.62	0.60	-2.97	-0.61	9,817	12,645	1.00
	zi_Intercept	1.17	1.02	-0.83	3.21	14,653	26,380	1.00
	Year2018	0.99	0.56	0.06	2.26	9.865	11,792	1.00
	zi_Year2018	-0.92	0.86	-2.55	0.84	9,123	20,453	1.00
	TreatSL	-0.55	0.79	-1.94	1.27	11,971	12,229	1.00
	zi_TreatSL	0.72	1.14	-1.53	2.97	30,828	36,467	1.00
	TreatSS	-1.31	0.94	-3.19	0.53	16,999	19,810	1.00
	zi_TreatSS	1.71	1.34	-0.84	4.42	24,519	34,493	1.00
	logVis	0.67	0.13	0.42	0.93	24,599	34,301	1.00
	zi_logVis	-1.74	0.41	-2.48	-0.86	21,776	27,539	1.00

|								
	Vegetation	1.51	0.27	0.98	2.04	19,506	28,285	1.00
	zi_Vegetation	-3.37	0.76	-4.91	-1.92	14,132	25,992	1.00
	Year18:TreatSL	-0.50	0.75	-2.22	0.81	12,180	10,793	1.00
	zi_Year18:TreatSL	0.30	0.97	-1.58	2.27	17,555	33,649	1.00
	Year18:TreatSS	1.79	0.90	0.03	3.62	17,246	18,784	1.00
	zi_Year18:TreatSS	-1.21	1.20	-3.60	1.13	19,854	34,447	1.00
Sand goby	Intercept	-0.39	0.18	-0.73	-0.04	693	279	1.01
MaxN	Year2018	-1.02	0.18	-1.38	-0.68	3,770	6,928	1.00
	TreatSL	0.23	0.27	-0.37	0.73	426	79	1.01
	TreatSS	0.25	0.23	-0.21	0.68	4,475	8,584	1.00
	logVis	1.30	0.09	1.13	1.48	780	1,147	1.01
	Vegetation	-1.79	0.18	-2.16	-1.45	3,388	9,036	1.00
	Year18:TreatSL	-0.77	0.26	-1.27	-0.27	1,421	8,217	1.00
	Year18:TreatSS	0.20	0.23	-0.23	0.64	1,293	7,602	1.01
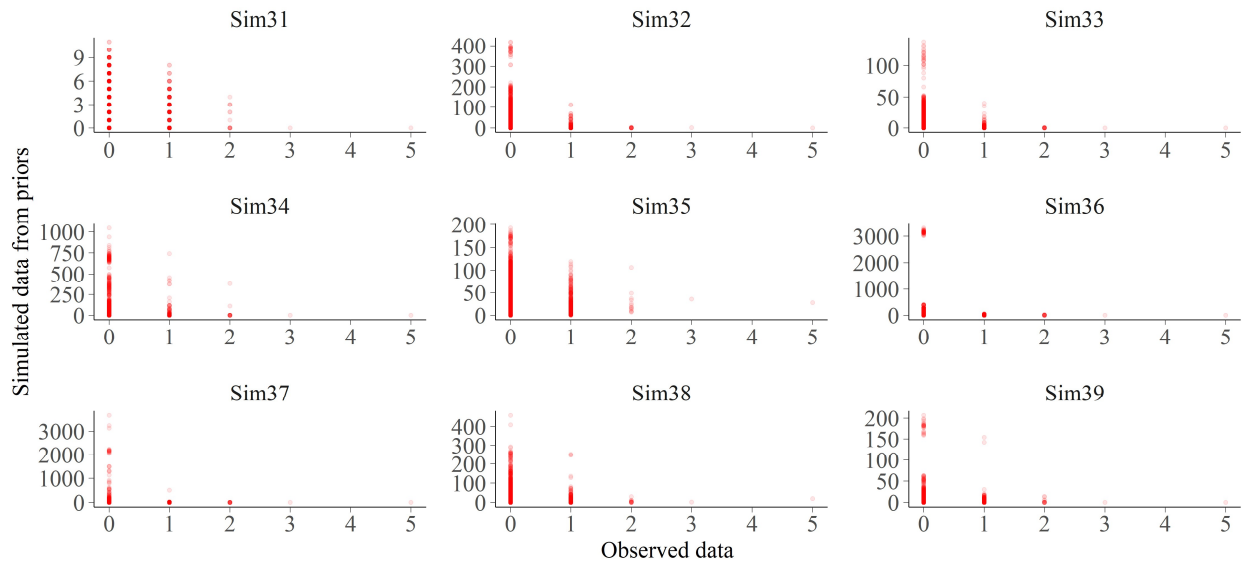
Figure S3.4 – Example of prior predictive checks for the flatfish model (see Table S3.2) following the protocol of Gabry et al. (2019). The example shows nine simulations out of 5000 simulated datasets from a model fitted on the selected final priors only (i.e. excluding the raw data). Simulated flatfish counts (MaxN) from the example datasets range from a maximum of 9 in Sim31 to counts of 3000+ in Sim36 and Sim37. The raw observations (horizontal axis) ranged from a MaxN of 0 to 5 only (i.e. a maximum of five flatfish observed in a single video frame). Simulations from the default priors (not shown here) reached MaxN counts of 10^7, which are unrealistic to be counted from video recordings by observers and cause unnecessary convergence issues when fitting the model on the data. Instead, the weakly informative priors selected for the flatfish model (Table S3.2) drastically improved computational efficiency (i.e. faster convergence and fewer divergent transitions) by assigning zero mass on extremely high (i.e. >10^6) counts from video recordings.

S3.4 – GLMLM diagnostics

A) Negative binomial distribution

B) Zero-inflated negative binomial distribution
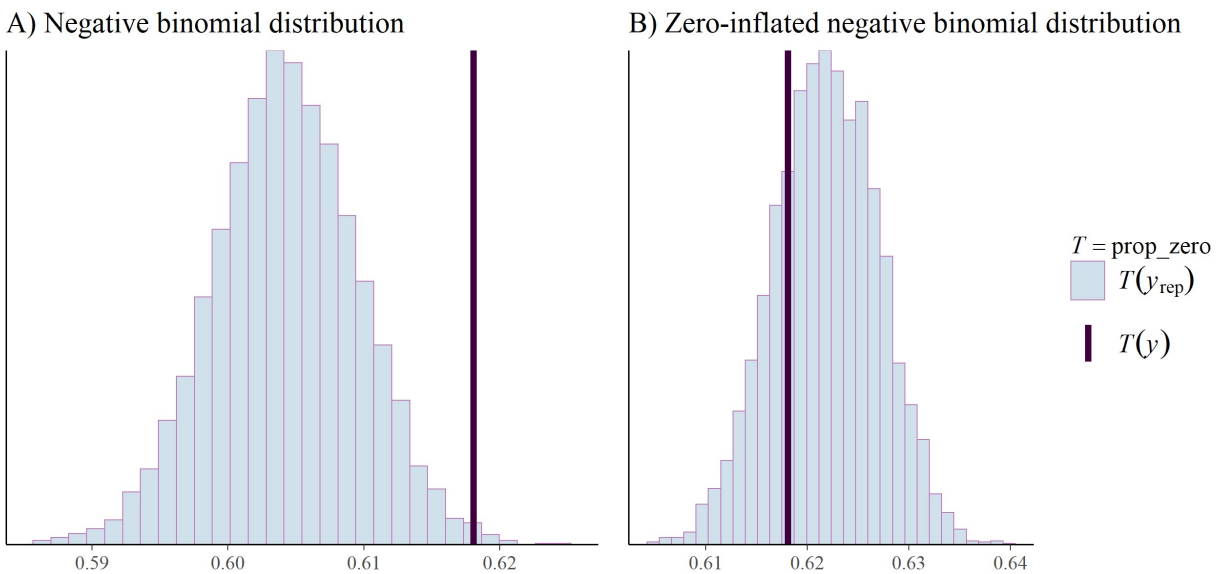
$T = \text{prop\_zero}$
$T(y_{\text{rep}})$
$T(y)$

Figure S3.5 – Example of a zero test for demersal goby abundance modelled using a negative binomial (NB2) distribution (A) and a zero-inflated negative binomial (ZINB) distribution (B). Light blue bars indicate the proportion of zeros in 5,000 simulated datasets, while the purple bar shows the proportion of zeros in the original dataset (approximately 61.8% of zeros). The test indicates that the large number of zeros is not adequately captured in the negative binomial model (i.e. slight zero-inflation). Adding a separate structure to the model to capture these excess zeros (and effectively convert the NB2 into a ZINB model) clearly improves the representation of zeros in the model.

A) Poisson distribution   B) Conway-Maxwell Poisson distribution

T = prop_zero
$T(y_{\text{rep}})$
$T(y)$
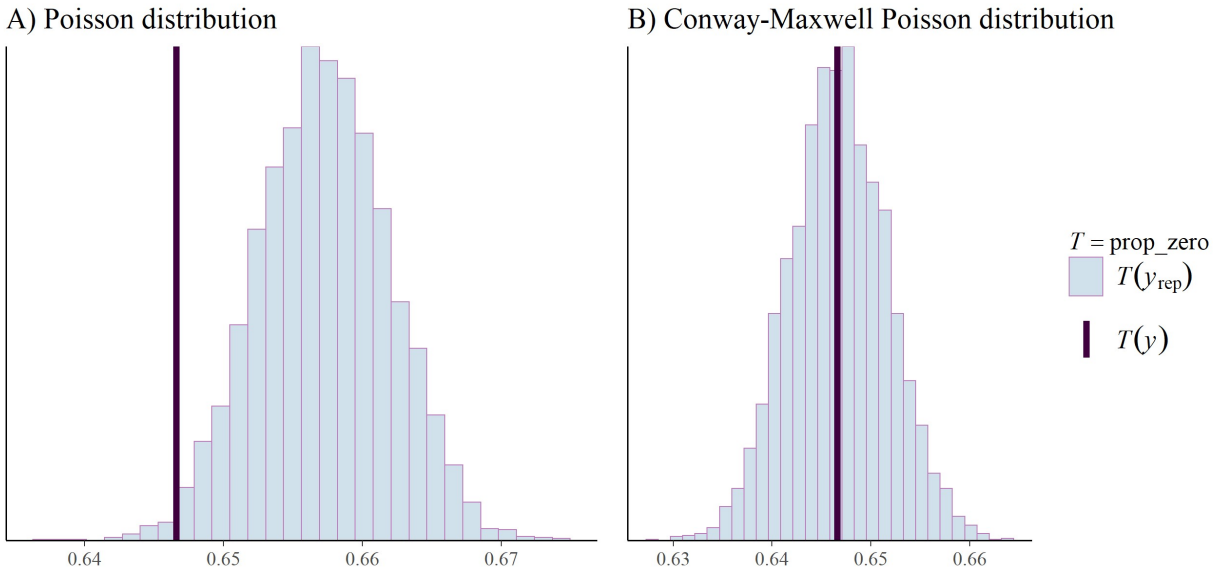
Figure S3.6 – Example of a zero test for sand goby abundance modelled using a Poisson distribution (A) and a Conway-Maxwell Poisson distribution (B). Light blue bars indicate the proportion of zeros in 5,000 simulated datasets, while the purple bar shows the proportion of zeros in the original dataset (approximately 64.7% of zeros). The test indicates that the number of zeros in the dataset is lower than expected under a Poisson model (i.e. zero deflation). Instead, fitting a Conway-Maxwell Poisson model can effectively deal with this zero-deflation and improve the overall model fit.

Figure S3.7 – Example of a dispersion test for gadoid abundance models. This example indicates overdispersion in the initial model following a Poisson distribution (A), i.e. excess variation in the data represented by the purple dot, which remains unexplained by the Poisson model assuming an equal mean and variance. The yellow cloud of dots shows the dispersion of 5,000 simulated datasets. Using a negative binomial distribution instead, in which the variance increasing quadratically with the mean, the dispersion of the data falls within the dispersion of the simulated datasets to improve the model fit.

A) Poisson distribution B) Conway-Maxwell Poisson distribution
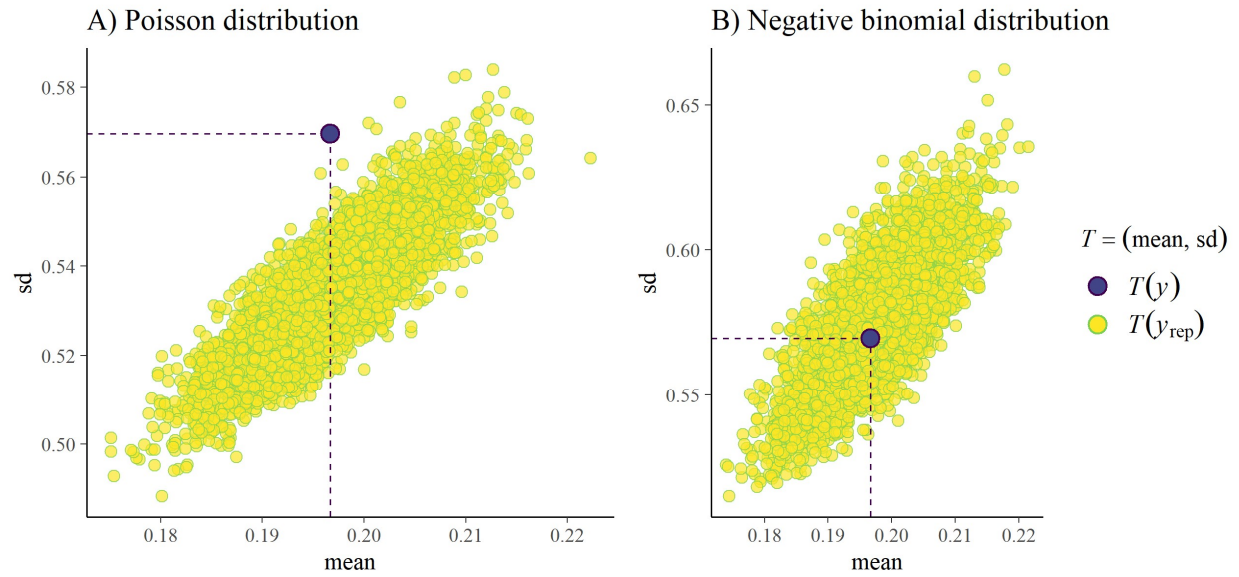
$T = (\text{mean, sd})$
● $T(y)$
○ $T(y_{\text{rep}})$
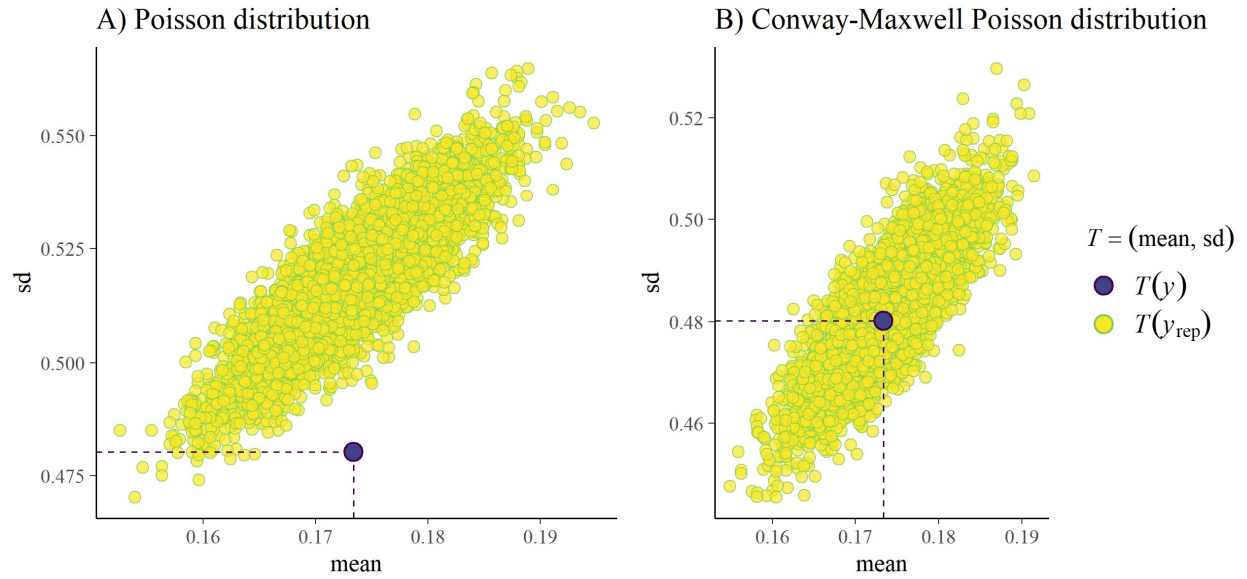
Figure S3.8 – Example of a dispersion test for labrid abundance models. This example indicates underdispersion in the initial Poisson model (A), i.e. less variation in the data represented by the purple dot, than is expected given a Poisson distribution. The yellow cloud of dots shows the dispersion of 5,000 simulated datasets. Using a Conway-Maxwell Poisson (CMP) model instead illustrates that the CMP distribution is effective at dealing with underdispersed counts (B), a phenomenon regularly encountered in ecological datasets.

A) Poisson distribution   B) Conway-Maxwell Poisson distribution
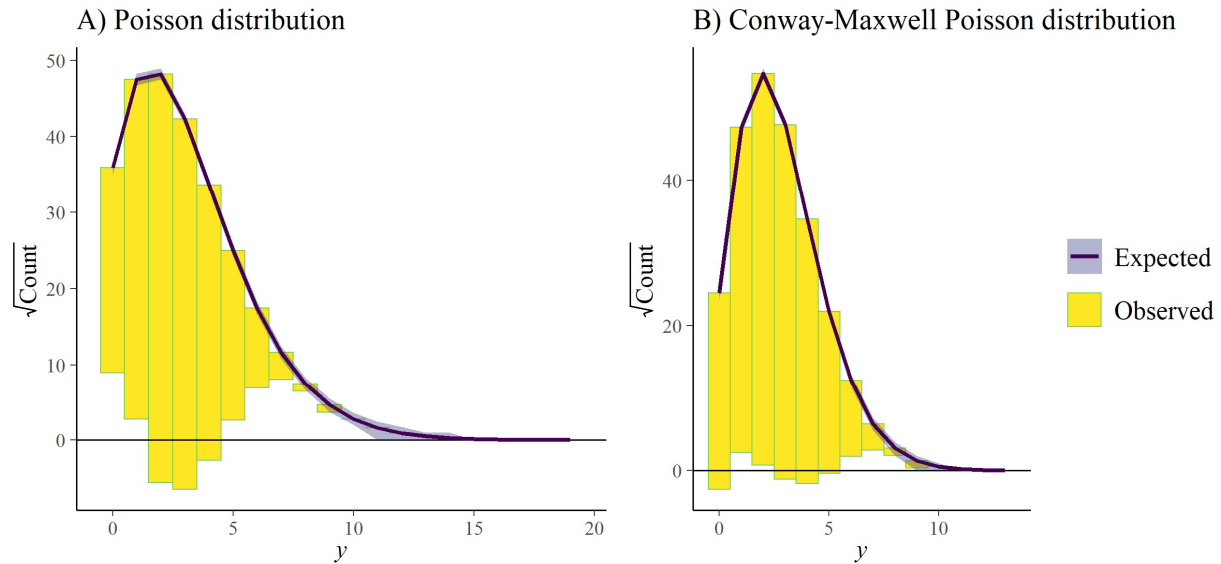
Figure S3.9 – Example of a hanging rootogram used to assess the overall fit with regard to the observed integer counts (here species richness). The hanging rootogram emphasizes the fitted values by aligning deviations along the horizontal axis (Kleiber & Zeileis, 2016). Therefore, large deviations of the observed data (yellow bars) from the horizontal reference line, especially in an undulating fashion, indicate a model misspecification. In the example above, changing the distribution from a Poisson to a Conway-Maxwell Poisson clearly improved the overall model fit. The initial Poisson model showed strong zero-deflation (i.e. the first yellow bar does not reach the reference line) together with two- and three-inflation (the third and fourth bar clearly surpass the reference line), which was largely resolved by fitting a Conway-Maxwell distribution to our data.

S3.5 – LVM diagnostics
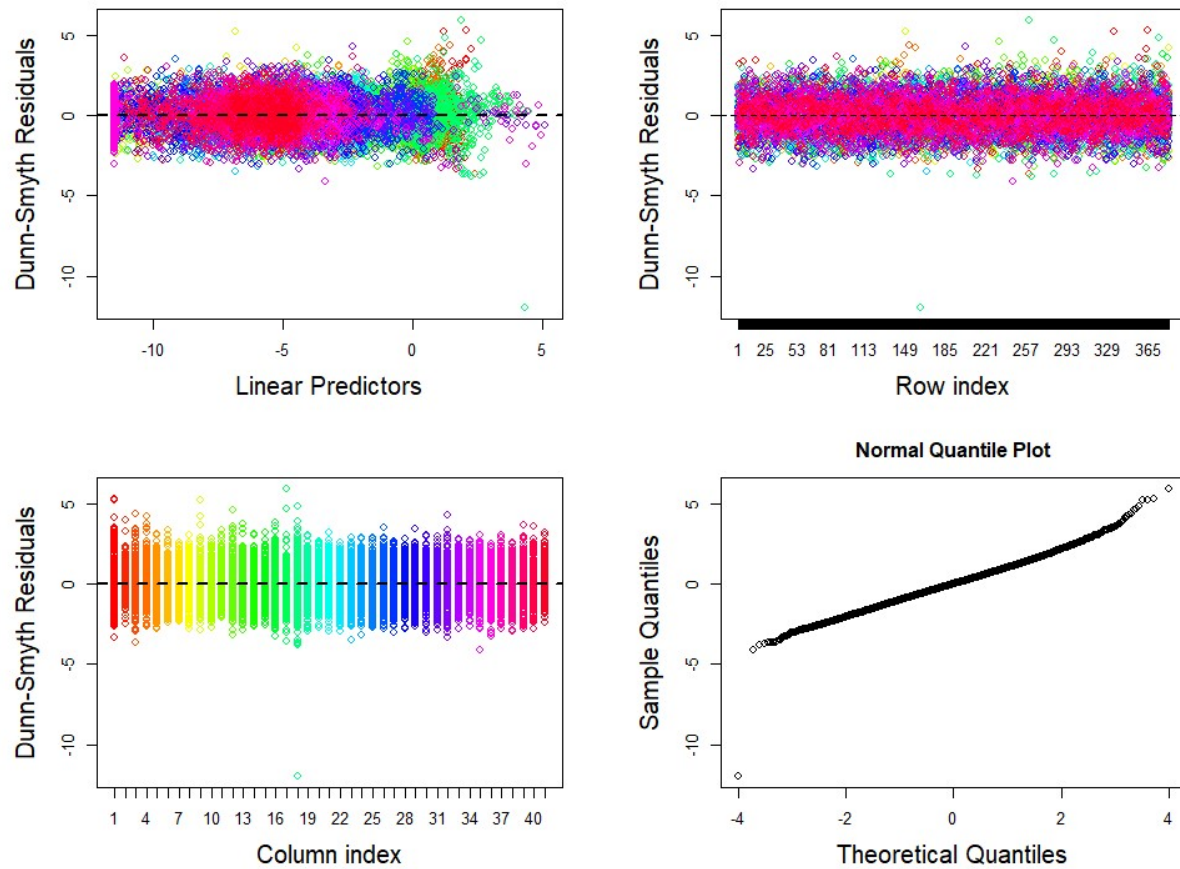
Figure S3.10 – Model diagnostic plots for a regular Poisson latent variable model (LVM). Note the presence of outliers in all the plots, a slight funneling effect in the top left plot (indicating potential overdispersion) and the unusual normal quantile plot suggesting that the Poisson distribution may not be appropriate given the dataset (Hui, 2016).
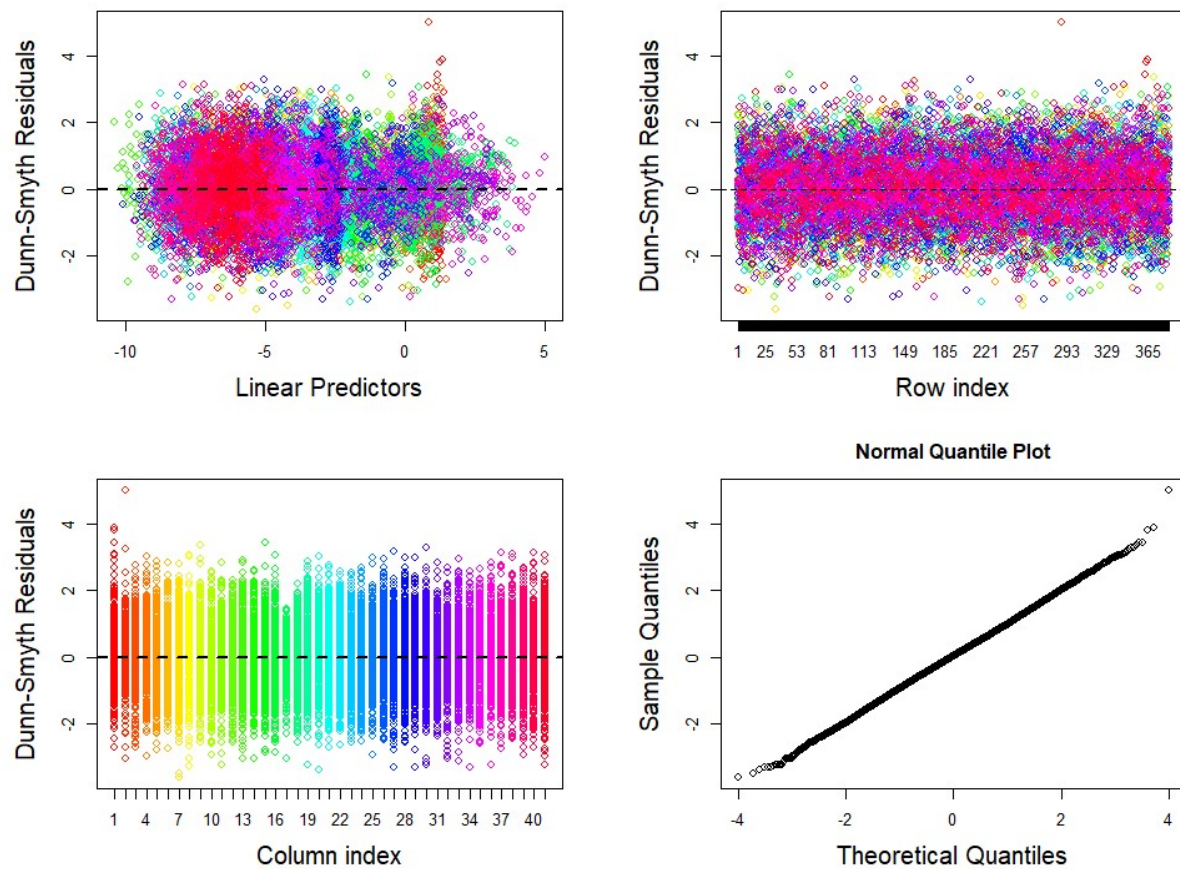
Figure S3.11 – Model diagnostic plots for a negative binomial LVM. The funneling effect in the top left plot is less apparent, outliers have disappeared and the normal quantile plot suggests a better distributional fit. These diagnostics were verified by comparing BIC and WAIC values between the two LVMs, which also indicated a strong preference for the negative binomial LVM.

S3.6 – Spatial distributions
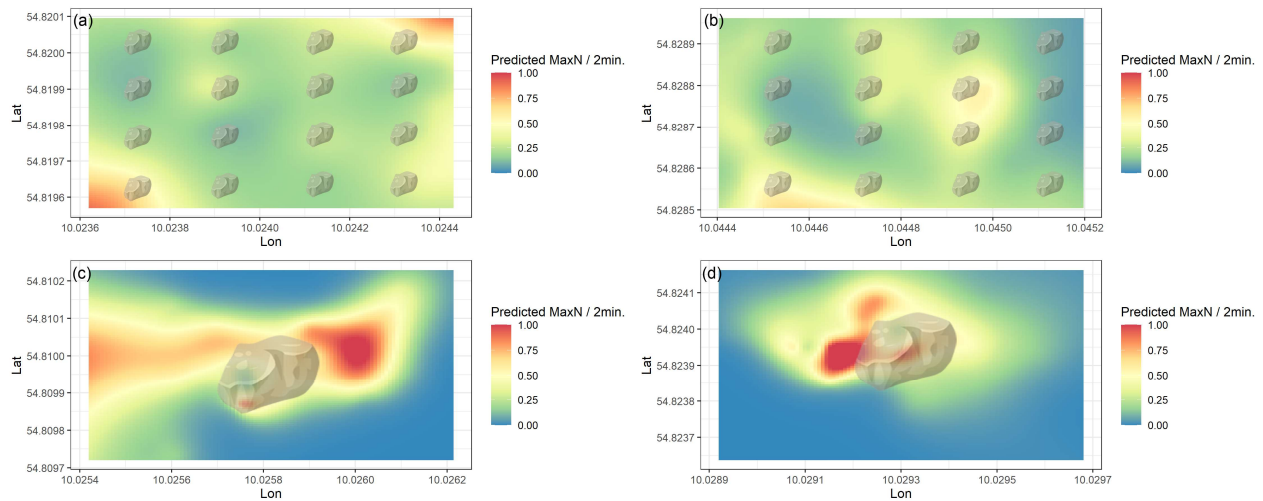
Figure S3.12 – Predicted spatial distribution of gadoids (*Gadidae spp.*) abundance at SS (a, b) and SL (c, d) sites. Model predictions are based on separate Generalized Additive Models (GAMs) for each field site, with a thin plate regression smoother on longitude-latitude coordinates of camera deployments in the field. Positions of the individual reef units are superimposed as semi-transparent boulders to allow for visualization of underlying model predictions. Note that positioning of the grids is based on coordinates of the outermost camera deployments, and therefore the relative positioning of the SL center within the grid is slightly different between the two SL sites. Model predictions were plotted on a grid using the 'STRbook' package (Wikle, Zammit-Mangion, & Cressie, 2019).
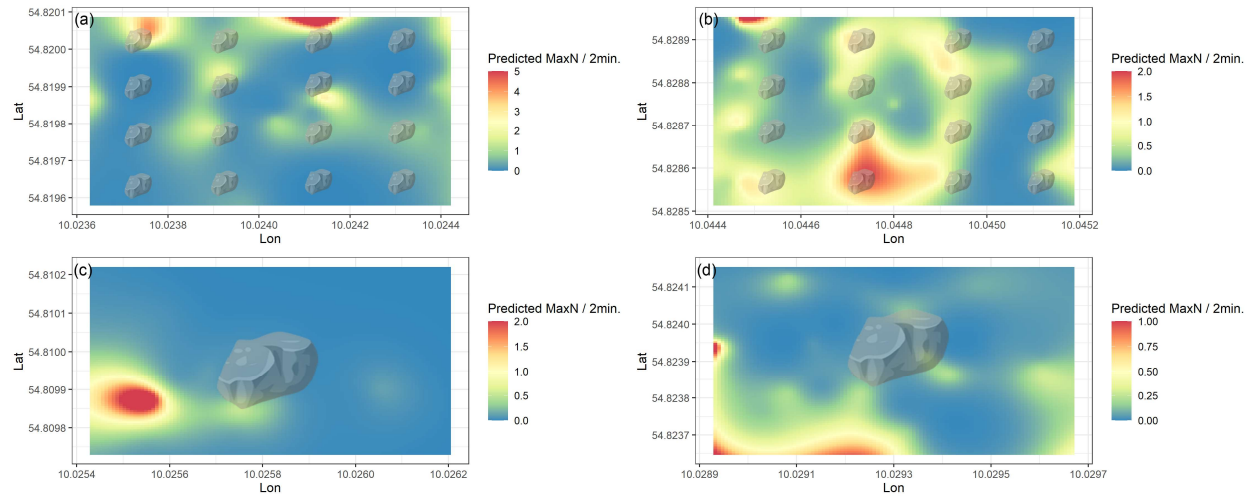
Figure S3.13 – Predicted spatial distribution of sand goby (*Gobiidae spp.*) abundance at SS (a, b) and SL (c, d) sites. See Fig. S3.12 for a detailed explanation. Note that the range of predictions was allowed to differ per field site to better highlight variations in predicted abundance at sites of low relative abundance (i.e. SL sites).

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Chapter 4

Comparing methodologies in marine habitat monitoring research: An assessment of species-habitat relationships as revealed by baited and unbaited remote underwater video systems*

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Abstract

Remote underwater video systems (RUVS) are increasingly used in scientific studies to monitor marine ecosystems. RUVS can be deployed baited (BRUVS) or unbaited (UBRUVS) and allow for an estimation of the MaxN metric, a relative measure of species abundance. Recording species MaxN in a variety of marine habitats provides associations between species abundance and habitat type, known as 'species-habitat relationships'. However, the introduction of bait in BRUVS could obscure such relationships by inadvertently attracting fish from neighbouring habitats. Here, we investigate the species-habitat relationships of seven temperate marine species: Atlantic cod (*Gadus morhua*), right-eyed flatfish (*Pleuronectinae spp.*), Atlantic herring (*Clupea harengus*), whiting (*Merlangius merlangus*), goldsinny wrasse (*Ctenolabrus rupestris*), two-spotted goby (*Gobiusculus flavescens*) and shore crab (*Carcinus maenas*) as revealed by BRUVS and UBRUVS. Video systems were deployed in coastal areas in the Western Baltic, with a cobble coverage ranging from 0% to 100%. Results show that all focal species responded to changes in cobble coverage, highlighting the importance of benthic habitat for a range of animals including benthopelagic species. Specifically, mean abundances of goldsinny wrasse and two-spotted goby were positively associated with cobble coverage, whilst right-eyed flatfish, herring, whiting and shore crab showed a negative association. Atlantic cod was found to be most abundant at intermediate cobble coverage between 65-70%, showing a unimodal trend with the mean abundance decreasing again at higher coverage. The relationships suggest that anthropogenic activities modifying benthic habitats, including extraction of material from the seabed and bottom trawling, likely change abundances of a range of marine species. Our comparative analysis suggests that both BRUVS and UBRUVS can identify previously documented species-habitat relationships. However, BRUVS demonstrated some superiority by confirming documented relationships for Atlantic cod and whiting, whilst UBRUVS failed to record any patterns for those species. Our data highlight the ability of BRUVS to identify changes in abundance across different habitats for a variety of species. Although the bait plume serves to attract organisms to the field of view, current results suggest that the use of BRUVS does not obscure species-habitat relationships in patchy coastal habitats. Therefore, future studies examining the importance of different marine areas may benefit from using BRUVS to quantify relationships between habitat variables and species abundance. Developing a better understanding of such relationships will be crucial in ensuring adequate management and protection of ecologically important marine habitats.

4.1. Introduction

Anthropogenic pressures on marine ecosystems have been recorded globally; highly impacted areas range from the North Sea and coastal areas of the Baltic Sea, to eastern Caribbean and Japanese waters (Halpern et al., 2008; Korpinen et al., 2012). Impacts can be due to direct exploitation of coastal resources, including overfishing and land reclamation of shallow-water habitats, but also indirect effects of rapid population growth on urbanisation and industry (Brown et al., 2018; Halpern et al., 2008; Korpinen et al., 2012; Lin and Yu, 2018; Pihl et al., 2006; Vasconcelos et al., 2007). Globally, directives have been put in place to protect marine habitats (European Parliament and Council of the European Union, 2008; Feng et al., 2016; Fernandes et al., 2005; UK Parliament, 2009). In the European Union, protection includes the establishment of 'Natura 2000' areas. The Natura 2000 legislation covers one of the largest protected areas in the world (Kristensen et al., 2017). Management of Natura 2000 areas varies from minor interventions such as reducing disruptive activities during breeding seasons, to major restoration works of degraded marine habitats in order to protect threatened species (Kristensen et al., 2017; Nature Agency, 2016). Surveys of these habitats allow for the assessment of changing habitat variables and associated marine community responses. These surveys generate important information including abundance data, commonly used to investigate the impacts of anthropogenic pressures (Bellwood et al., 2012; Hillebrand et al., 2018; Stallings, 2009), as well as the influence of natural habitat components, such as vegetation (Alós et al., 2018) and habitat complexity (Bell et al., 1987; Watson et al., 2005) on fish populations. Specifically, in terms of habitat restoration and management, long-term monitoring is required to examine the ecological responses to management measures and assess the efficacy of the restoration efforts. Ultimately, this helps inform future restoration strategies and marine spatial planning (Lester et al., 2018; McHenry et al., 2017; Pınarbaşı et al., 2017).

Habitat complexity encompasses size, shape and texture of seafloor features (Connell and Jones, 1991; Gee and Warwick, 1994). For example, rocky seafloors have a higher habitat complexity than sandy bottoms (Kristensen et al., 2017). Past findings have indicated that marine environments with high habitat complexity often support greater abundance of individual species than less complex environments (Campbell et al., 2018; Connell and Jones, 1991; Green et al., 2013; Jenkins and Wheatley, 1998). However, there are examples of limited (Almany, 2004) or no influence (Roberts and Ormond, 1987) of habitat complexity on species abundance indicating that some species do not exhibit this preference for high complexity. Importantly, it is still unclear to what extent different methodologies used to investigate fish abundance produce comparable results (Cappo et al., 2004, 2003; Cundy et al., 2017; Willis and Babcock, 2000).

The umbrella term RUVS (remote underwater video systems) is used to describe underwater recording techniques, including Baited RUVS (BRUVS) and Unbaited RUVS (UBRUVS). RUVS can be limited by poor water visibility and can make the observation of cryptic fish species challenging, particularly in complex habitats (Hannah and Blume, 2012; Watson et al., 2005). However, RUVS have a significant advantage because they are non-extractive and less damaging to complex seafloors, compared to many netting and trawling methods (Ebner and Morgan, 2013; Morrison and

Carbines, 2006; Willis and Babcock, 2000). This is crucial for sampling in protected sites such as Natura 2000 areas, where reefs are listed as an important habitat type for conservation (Nature Agency, 2016). Because of the minimised impact on benthic habitats, BRUVS have been used regularly to investigate the effectiveness of designated marine reserves such as 'no take' areas (Coleman et al., 2015; McLaren et al., 2015), as well as the spatial distribution of species across a variety of marine habitats (Espinoza et al., 2014; Langlois et al., 2012; White et al., 2013). BRUVS are considered an effective way to study species abundance because the bait attracts fish to the field of view (FOV), thus allowing for accurate counts and identification (Hardinge et al., 2013; Watson et al., 2005). BRUVS document not only species attracted to the bait itself, but also those that pass the FOV by chance or are attracted to the general activity surrounding the bait (Harvey et al., 2007). The overall attraction means BRUVS generally sample more fish, which reduces the variance of species abundance per habitat, and increases statistical power (Harvey et al., 2007).

Although many studies have used BRUVS, there are some concerns surrounding bias in the fish attracted to BRUVS (Whitmarsh et al., 2017). Firstly, BRUVS may attract a disproportionate number of carnivorous fish species compared to herbivores (Bernard and Götz, 2012; Colton and Swearer, 2010; Watson et al., 2005; Wraith et al., 2013). Secondly, BRUVS may yield an overrepresentation of larger specimens because they forage further, or demonstrate dominance and predatory behaviour towards smaller fish (Hardinge et al., 2013; White et al., 2013). Finally, fish attracted to the bait may originate from a neighbouring habitat (Hannah and Blume, 2012). Overall, these biases imply there is a risk that the relationship between a habitat variable and a certain species' abundance (henceforth termed 'species-habitat relationships') may be obscured by BRUVS.

UBRUVS offer an alternative underwater video method which may produce more robust species-habitat relationships and provide more accurate representations of the fish population because there is no bait plume (Colton and Swearer, 2010; Hannah and Blume, 2012; Harvey et al., 2007). However, fewer studies use UBRUVS, mainly because they rely solely on fish passing the FOV by chance. Consequently, species abundances recorded by UBRUVS have been consistently lower than those sampled by BRUVS (Cappo et al., 2006; Hardinge et al., 2013; Watson et al., 2005). Therefore, a larger number of UBRUVS site replicates are needed to ensure adequate statistical power (Watson et al., 2005). Increased deployments inevitably increase the costs associated with fieldwork, as well as the time required to complete data collection and video analysis.

We developed species-habitat relationships for seven temperate marine species in the Western Baltic Sea and compared the direction of these relationships as recorded by BRUVS and UBRUVS. Specifically, the hypotheses tested were that: 1) species abundance varies with respect to benthic cobble coverage, ranging from 0 to 100% cobble; 2) the directions of the species-habitat relationships are comparable between the two sampling methods and 3) UBRUVS record more robust species-habitat relationships than BRUVS due to the obscuring effects of BRUVS.

4.2. Materials and Methods

4.2.1. Study area

Sampling was carried out between April and May 2017 in the Sønderborg Bay area of the Flensborg Fjord (Fig. 4.1), situated in the Western Baltic, between Denmark and Germany. A total of six sites were sampled: two cobble reef habitats (Vesterhage and Spar Es) and four sand habitats (Vemmingbund, Kegnæs Ende, Sønderskov and Dybbøl Mølle). In each of the six sites, sampling took place within a 200 m wide and 500 m long rectangular area along the coastline to ensure standardisation of both sampling area and water depth. All sampling was carried out at depths ranging between 6 and 7 m.

4.2.2. RUVS setup and deployment

GoPro cameras (Hero 3, 3+ and 4; www.gopro.com) were used for both BRUVS and UBRUVS. All cameras were set with identical video settings including 720p video resolution and 30 frames per second (NTSC). Cameras used for UBRUVS were equipped with intervalometers (model: Time Lapse Intervalometer or BlinkX, CamDo Solutions; www.cam-do.com). All intervalometers were programmed such that every hour the UBRUVS would turn on, record for 2 minutes, and then turn off. Due to the current study being part of a larger monitoring program with various aims, this setting was applied to ensure that all UBRUVS recorded for more than 24 hours and data would cover the entire diel cycle. In contrast, BRUVS started recording immediately after deployment and ran continuously. BRUVS were therefore equipped with an extra battery (BacPac; www.gopro.com) to ensure adequate recording time to capture the bait plume effect (Harasti et al., 2015). Accordingly, BRUVS recorded for a minimum of 40 minutes and up until 2 hours, whilst UBRUVS recordings exceeded 24 hours and typically ran for 40 hours (recording 2 minutes per hour). Standard GoPro waterproof housing held the cameras in position.

The RUVS setup consisted of a metal pole (3 cm diameter; 100 cm high) fixed to a concrete base (45 x 45 x 5 cm; length x width x height). The camera was secured horizontally to the pole at a height of 20 cm above the seafloor. Below the cameras used for BRUVS, an 80 cm long bait arm was attached to hold a mesh bait bag containing 500g of fresh Atlantic herring (*Clupea harengus*) bait chopped into 1-2 cm chunks. Each 10 cm increment along the bait arm was marked with tape for visibility estimates (Fig. 4.2a; Cappo et al., 2003). UBRUVS setup resembled that of BRUVS, but instead of a bait arm, a rope was attached to the concrete base and extended horizontally in the camera's FOV. The rope lay flat along the seafloor, kept taut by an anchor at the end. Metre markers were attached to the rope and used for visibility estimates (Fig. 4.2b).

Deployment procedure resembled previous studies (for detailed account see Langlois et al., 2018). Similar to Gilby et al., (2017), there was a maximum of two temporally overlapping deployments (either two BRUVS or two UBRUVS) at each of the six sites (Fig. 4.1). BRUVS and UBRUVS could run concurrently if they were at separate sites, but they were never run at the same time within a single site. Seafloors with either mainly sand (Fig. 4.2b) or cobble (Fig. 4.2c) were identified within each site using a side scanner (Lowrance Elite-7 Ti; www.lowrance.com). The side

scanner was used to confirm there was at least 25 m diameter of the desired habitat (sand or cobble or a mix) for each camera deployment. In those cases where two BRUVS or two UBRUVS were running
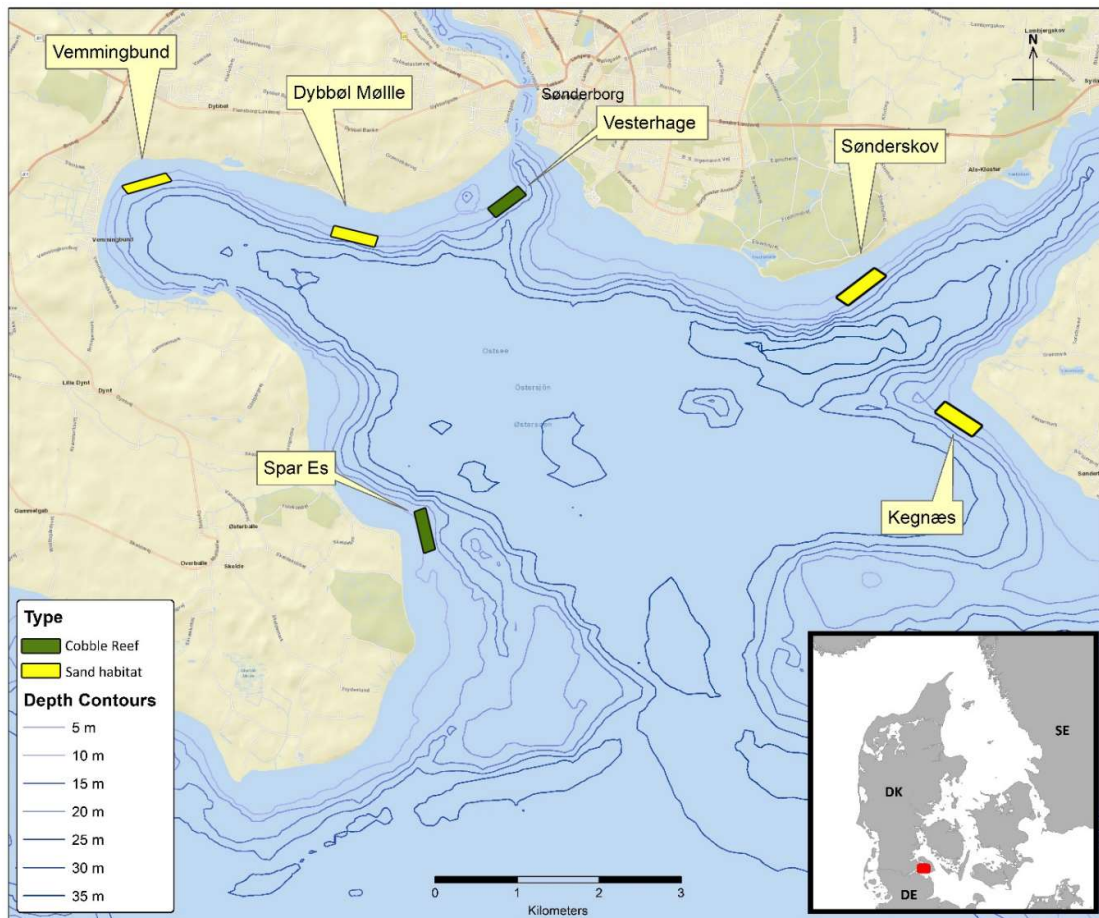
Figure 4.1 – Sønderborg Bay in southern Denmark where the six study sites are indicated. Underwater video systems were deployed within rectangular areas along the coast to ensure a water depth between 6 and 7m. The bay is dominated by either cobble reef (green rectangles) or sand bottom habitats (yellow rectangles), however, scattered cobble reef occurs intermittently in the sand habitat and *vice versa*, reflecting the mosaic nature of the seabed in Sønderborg Bay.

simultaneously within a site, the distance between the two systems depended on the available habitat and ranged between 50 – 450 m (comparable to e.g. Hesse et al., 2016; Wakefield et al., 2013). Notably, all deployments were conducted during daylight hours and BRUVS were deployed at least 2 hours before sunset to ensure adequate light levels for the continuous recordings. Video samples in complete darkness, i.e. produced by UBRUVS that had continued recording into the night, were discarded. Day lengths varied between 13 and 17 hours during the study period. Accordingly, a total of 293 deployments were identified as successful based on these criteria, consisting of 141 BRUVS

and 152 UBRUVS deployments. The UBRUVS deployments in turn produced 2,621 video samples between all locations (Table 4.1), each with a recording time of two minutes.

Figure 4.2 – Multi-pane overview of the RUVS used for monitoring in this study. A) BRUVS setup with a bait arm extended within the FOV, marked with tape at 10 cm increments used for visibility estimates, B) Sand site with low cobble coverage recorded by a BRUVS, C) Reef site with high cobble coverage, recorded by an UBRUVS with a sponge attached to a rope at 1 m distance from the camera for visibility estimates and D) a school of Atlantic cod (*Gadus morhua*) passing the FOV, recorded at intermediate cobble coverage by a BRUVS at one of the reef sites.

4.2.3. Video analyses

Recordings were analysed using the VLC Media Player (VideoLan; www.videolan.org). Video analysis revealed the species present and the maximum number of each species in a frame at one time, the MaxN metric (Cappo et al., 2004; Ellis and DeMartini, 1995; Priede and Merrett, 1996). The use of MaxN eliminates the risk of repeated counts of the same individuals re-entering the FOV and provides a conservative estimate of species abundance (Campbell et al., 2018; Colton and Swearer, 2010; Harvey et al., 2007; Watson et al., 2005). Individuals were ideally classified to species level using compiled reference images, as well as by consulting colleagues with expertise in specific fish families. Following Cundy et al. (2017) and Hardinge et al. (2013), where species identification was not possible, individuals were assembled in groups by using the lowest common taxonomic level possible. Most notably for this study, flounder (*Platichthys flesus*), dab (*Limanda limanda*) and plaice (*Pleuronectes platessa*) were grouped under the common sub-family of right-eyed flatfish, *Pleuronectinae*. There were only few sightings for certain species, so it was decided to focus the analysis on species for which there were a sufficient number of observations (Campbell et al., 2018;

Unsworth et al., 2015). Accordingly, we analysed the abundances of Atlantic cod ("cod"; Fig. 4.2d; *Gadus morhua*), right-eyed flatfish ("flatfish"; *Pleuronectinae spp.*), Atlantic herring ("herring"; *Clupea harengus*), whiting (*Merlangius merlangus*), goldsinny wrasse (*Ctenolabrus rupestris*), two-spotted goby (*Gobiusculus flavescens*) and shore crab (*Carcinus maenus*).

Table 4.1 – Successful BRUVS and UBRUVS replicate deployments and individual sampling units with respect to the field sites (see Fig. 4.1).

Location	Habitat type	BRUVS Deployments	UBRUVS Deployments	2-minute samples
Dybbøl Mølle	Sand	25	27	469
Kegnæs	Sand	25	24	262
Spar Es	Reef	24	24	636
Sønderskov	Sand	22	27	337
Vemmingbund	Sand	21	28	492
Vesterhage	Reef	24	22	425

Ultimately, for each sampling unit, data were collected on the species present (or lowest taxonomic level), MaxN, cobble coverage (%), functional visibility (m) and FOV obstruction (%; abbreviated FOV Ob). Functional visibility was defined as the furthest distance in which a fish would still be identifiable; this varied with water turbidity and was estimated using the bait arm (BRUVS) or rope markers (UBRUVS). Obstruction of the FOV was estimated using the percentage of seabed structures or macroalgae rising above the seafloor, and obstructing the view, within the distance of the functional visibility. Cobble coverage (%) was assessed using the video footage by estimating the percentage of the visible seafloor which was made up of cobble. Cobble is defined as 'a heterogeneous mixture of pebbles, cobbles, and boulders' (Shepard, 1963; Wahle and Steneck, 1992). Specifically, the size range for cobble is classed as rocks between 6-26 cm diameter (Greene et al., 1999; Hannah and Blume, 2012; Wahle and Steneck, 1992). Occasionally, a boulder (diameter > 26 cm) was present in the FOV and included in the cobble coverage. Cobble coverage was then used as an explanatory variable in the analysis to model the species-habitat relationships.

4.2.4. Statistical analysis

We used generalised linear mixed models (GLMMs) to analyse the effect of cobble coverage (CC) on the abundance (MaxN) of the seven focal species of this study. Additionally, to investigate whether any of the focal species showed a unimodal relationship with cobble coverage, we included a quadratic term of this predictor (CC^2) in the models. Following from the sampling design, a nested error structure was included in the UBRUVS models, with deployment ID (a unique ID assigned to each deployment) being nested in site ID. This allowed the models to incorporate potential correlations between sampling units from the same deployment, as well as between deployments

coming from the same field site. For BRUVS models, we instead used the single random effect of site ID since sampling units consisted of whole deployments. In addition, we defined the soak time (i.e. total recording time) of BRUVS as an exposure term, by adding the logarithm of soak time as a predictor in the model (Zuur and Ieno, 2016) to account for differences in recording time. Functional visibility and FOV obstruction were included as additional covariates for both sampling methods, since these two variables potentially indirectly influenced results by altering the area size in the FOV. Model selection was performed using a backward elimination routine based on Akaike Information Criterion (AIC) values. This involved comparing the full model (i.e. the polynomial regression model) with nested models containing a relevant subset of predictors. In the case of ΔAIC-values less than 2, implying substantial empirical support for either model (Burnham and Anderson, 2002), we selected the most parsimonious model (i.e. the linear regression model) whilst retaining the effects of differences in exposure (soak time, BRUVS only) and FOV. Models were fitted using a log-link function with the response variable following either a Poisson or negative binomial distribution. Since none of the models showed signs of overdispersion, the choice of the distribution was instead based on comparison of the AIC values.

As highlighted by Colton and Swearer (2010), comparison between methodologies is often confounded by differences in the data collection process. In this study, sampling units for BRUVS and UBRUVS differed in recording time, due to the data collection process being conducted as part of multiple monitoring studies with various aims. Since this precludes a direct comparison between species abundances as sampled by the two methods, we used separate GLMMs for BRUVS and UBRUVS instead of including sampling method as a predictor in a pooled model.

Data were analysed in R version 3.4.4 (R Development Core Team, 2014) using package glmmTMB version 0.2.1.0 (Brooks et al., 2017).

4.3. Results

4.3.1. Species-habitat relationships

The effect of cobble coverage on cod (*Gadus morhua*) abundance was found to be dependent on the sampling method. Specifically, BRUVS identified a unimodal relationship between cod abundance and cobble coverage (Fig. 4.3a; quadratic term in Table 4.2, $p < 0.05$), with the number of cod increasing until a tipping point between 65-70% coverage and slightly decreasing again at higher coverage. In contrast, UBRUVS did not record any significant cobble effect on mean cod abundance across the interval from 0% to 100% cobble coverage (Fig. 4.4a; Table 4.2; $p > 0.1$). For the subfamily of right-eyed flatfish (*Pleuronectinae spp.*), both sampling methods documented a similar species-habitat relationship (Table 4.2). Results show that increasing cobble coverage led to a significant reduction in mean flatfish abundance as recorded by BRUVS (Fig. 4.3b; $p < 0.05$) and UBRUVS (Fig. 4.4b; $p < 0.01$). This negative trend was found across the entire cobble coverage range without any evidence for unimodality recorded by the two sampling methods (Table 4.2).

Table 4.2 – GLMM parameter estimates for each of the seven focal species using either a Poisson or negative binomial (NB) distribution. Separate models were used for the two different sampling methods (BRUVS and UBRUVS). Significant effects (p < 0.05) of cobble coverage (CC) and the quadratic term (CC^2) are highlighted in bold, with the latter denoting a unimodal relationship between species abundance and cobble coverage. Visibility and FOV Obstruction (FOV Ob) were included as additional covariates. [excl. = excluded (based on AIC), N.A. = not applicable, (*) = $p < .1$; * = $p < .05$; ** = $p < .01$, *** = $p < .001$.]

Species	Common name	Sampling Method	Distri-bution	Intercept	CC	CC^2	Visibility	FOV Ob	log Soak
Gadus morhua	Atlantic cod	BRUVS	NB	-1.57	**5.67****	**-4.12***	1.10(*)	1.57	0.98
		UBRUVS	NB	-2.50	0.56	excl.	0.41(*)	-3.48(*)	N.A.
Pleuronectinae spp.	Flatfish	BRUVS	NB	-4.14	**-8.84***	excl.	2.93*	excl.	0.77
		UBRUVS	Poisson	-4.14	**-4.20****	excl.	0.67*	-1.14	N.A.
Clupea harengus	Atlantic herring	BRUVS	NB	2.74	-1.34(*)	excl.	excl.	excl.	0.94
		UBRUVS	NB	-5.10	-1.94	excl.	2.93***	-7.70	N.A.
Merlangius merlangus	Whiting	BRUVS	NB	0.77	**-2.40*****	excl.	0.61	excl.	0.76
		UBRUVS	NB	-2.89	-28.83	26.92	-0.69	-13.98	N.A.
Ctenolabrus rupestris	Goldsinny wrasse	BRUVS	Poisson	-3.16	**7.62*****	**-4.47 ***	0.69	1.38*	0.10
		UBRUVS	Poisson	-8.02	**3.39*****	excl.	excl.	excl.	N.A.
Gobiusculus flavescens	Two-spot goby	BRUVS	NB	-8.12	**11.72*****	**-6.10***	2.07**	excl.	1.33
		UBRUVS	NB	-10.19	**5.19*****	excl.	1.51***	2.61	N.A.
Carcinus maenas	Shore crab	BRUVS	NB	1.92	**-3.49****	**2.30***	-0.45	excl.	0.96***
		UBRUVS	Poisson	-3.91	**-14.06***	**11.93***	0.74**	-4.22	N.A.

The effect of cobble coverage on the abundance of Atlantic herring (*Clupea harengus*) was less pronounced. BRUVS revealed a decreasing trend of herring abundance with increasing cobble coverage (Fig. 4.3c), yet this relationship was not found to be statistically significant at the 95% level (Table 4.2; p < 0.1). In addition, no significant effects of cobble were recorded by UBRUVS despite a slightly decreasing trend in predicted herring abundance across cobble coverage (Fig. 4.4c). For whiting (*Merlangius merlangus*), BRUVS documented a negative association with cobble coverage (Fig. 4.3d; Table 4.2; p < 0.001). This relationship was found to be linear, with no evidence of a tipping point at higher ranges of cobble coverage. In contrast, UBRUVS recorded a weakly defined

relationship for whiting with no significant effect in the linear and quadratic terms of cobble coverage (Table 4.2). This lack of a clear habitat association for whiting was likely due to no individuals being recorded by UBRUVS at an intermediate cobble coverage, which was also apparent from the widening confidence band across these intermediate values (Fig. 4.4d).

The goldsinny wrasse (*Ctenolabrus rupestris*) showed a positive association with cobble, as evidenced by recordings from both sampling methods (Table 4.2; $p < 0.001$ for both methods). BRUVS documented a unimodal relationship with wrasse abundance peaking at 85-90% cobble coverage (Fig. 4.3e), whilst UBRUVS showed a linear increase in wrasse abundance across the range of cobble coverage (Fig. 4.4e; $p < 0.001$). Similarly, two-spotted goby (*Gobiusculus flavescens*) clearly increased in abundance from sand to reef habitat. Results from the BRUVS model showed a significant effect for the quadratic term of cobble coverage (Table 4.2; $p < 0.05$) with an increase in goby abundance up until 85% coverage after which goby numbers plateaued (Fig. 4.3f). This trend was absent in UBRUVS, which instead showed a linear increase across the range of cobble coverage (Fig. 4.4f; $p < 0.001$).

Finally, both sampling methods documented a negative unimodal relationship between shore crab (*Carcinus maenas*) abundance and cobble coverage (quadratic term in Table 4.2; $p < 0.05$ for both methods). Shore crabs were most abundant on sandy bottoms low in cobble coverage, decreasing steadily in abundance at the lower-intermediate range before slightly increasing again at higher coverages (Fig. 4.3g). However, similar to the results for whiting, UBRUVS recorded few shore crabs at an intermediate cobble coverage, causing increased uncertainty in the model predictions across these intermediate values (Fig. 4.4g).

4.3.2. Visibility and FOV obstruction

A number of effects were observed when accounting for visibility, which varied between species and sampling method. For flatfish and two-spotted goby there was a significant positive effect of visibility on the mean abundance documented by both methods, whilst an effect was only found in UBRUVS for herring and shore crab (Table 4.2). No significant influence of visibility was found in the models for whiting and goldsinny wrasse ($p > 0.1$). The FOV obstruction appeared to have little effect on the output of the models. Apart from a positive effect on the number of goldsinny wrasse recorded by BRUVS (Table 4.2; $p < 0.05$) and a negative (but not statistically significant) effect on the abundance of cod ($p < 0.1$), the FOV obstruction did not influence the results for the focal species in this study.
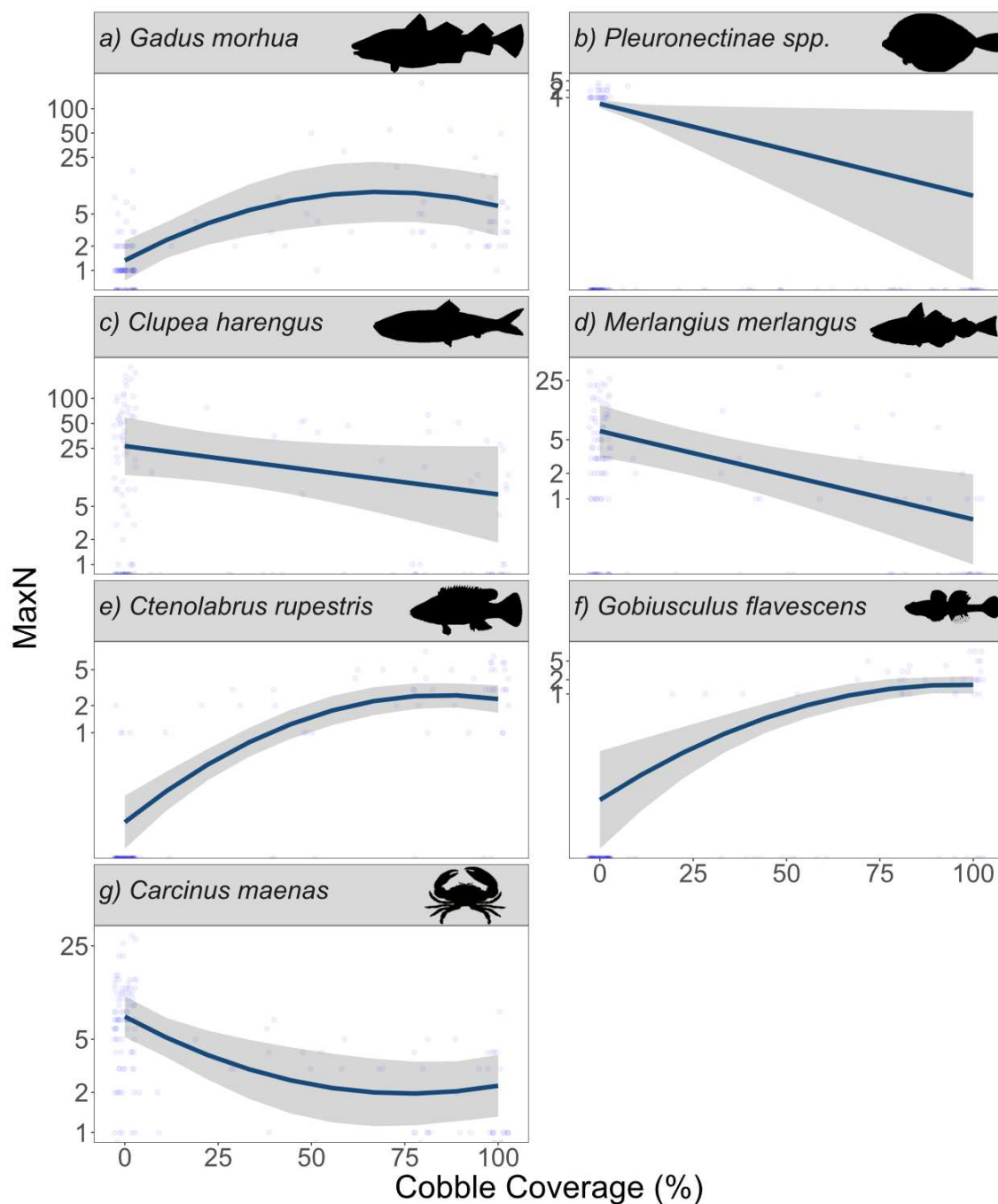
Figure 4.3 – Observed and predicted abundances (MaxN) recorded by BRUVS across varying cobble coverage. Distances between MaxN values on the y-axis were log-transformed for improved visualisation of the relationships. Solid lines show the mean GLMM predictions and the shaded areas represent the 95% confidence intervals. The blue dots show the original observations, which were jittered to improve visual representation. Results are shown for each of the focal species: (a) Atlantic cod (*Gadus morhua*); (b) Flatfish (*Pleuronectinae* spp.); (c) Atlantic herring (*Clupea harengus*); (d) Whiting (*Merlangius merlangus*); (e) Goldsinny wrasse (*Ctenolabrus rupestris*); (f) Two-spotted goby (*Gobiusculus flavescens*) and (g) Shore crab (*Carcinus maenas*).

4.4 Discussion

Using two different underwater video methods, BRUVS and UBRUVS, this study examined changes in the abundance of seven temperate marine species in habitats with varying cobble coverages. For all species, the mean abundance changed with cobble coverage, revealing the importance of benthic habitats for a variety of organisms including benthopelagic species. The identified relationships indicate that changes in cobble coverage, whether caused by human activity or natural events, could lead to a shift in the abundance of a range of marine species. Notably, we examined whether the use of bait in underwater video monitoring could bias associations between species abundance and habitat type, by potentially attracting individuals from greater distances and different habitats (Hannah and Blume, 2012). Our results reveal that BRUVS and UBRUVS were equally capable of identifying directions of species-habitat relationships for four out of the seven focal species in this study (flatfish, wrasse, goby and shore crab; Table 4.2). BRUVS recorded a significant relationship for an additional two species, cod and whiting, whilst UBRUVS failed to capture a significant habitat association for these species. This suggests that BRUVS did not obscure species-habitat relationships, but may in fact reveal more robust relationships compared to UBRUVS.

Following the concept of an inherent trade-off between environmental heterogeneity and the amount of habitat available to individual species (Allouche et al., 2012), we tested for unimodality within the species-habitat relationships. The abundance of a particular species may potentially be maximised or minimised at an intermediate cobble coverage, instead of increasing or decreasing continuously with coverage. Accordingly, a quadratic term for cobble coverage (CC^2) was included in all initial models and assessed for its significance (Table 4.2). The importance of considering unimodal patterns between fish community metrics and habitat complexity was recently highlighted for natural and artificial temperate reefs (Paxton et al., 2017). The authors reported that fish abundance was maximised at intermediate reef complexity, rather than increasing across the entire interval from low to high complexity. This pattern was observed both for artificial and natural temperate reefs. In the current study, the abundance of shore crab was found to be minimised at intermediate cobble coverage, whilst the abundance of cod peaked across this intermediate range. Shore crabs appear to be highly adaptable, able to populate diverse habitats including sand, mud and rocks (Cohen et al., 1995; Grosholz and Ruiz, 1995), which was also reflected by their presence at both sand and rocky sites in our study. Cod increased in abundance up to 65-70% of cobble coverage and slightly decreased again at higher coverage. This pattern suggests that cod abundance peaked in habitats of intermediate composition, e.g. reef edges or mosaics of hard and soft bottoms. Such habitats may offer elevated prey resources for cod by allowing the fish to include sand bottoms for foraging, whilst remaining in close proximity to reef structures for refuge against predation (Posey and Ambrose, 1994; Rosemond et al., 2018). In fact, since young cod largely prey on crustaceans (Pihl, 1982) and individuals recorded in our study typically were of subadult size, we cannot rule out the possibility that the low number of crabs recorded at intermediate cobble coverage is a direct consequence of maximised cod abundance at intermediate cobble coverage. Notably, unimodality was only recorded
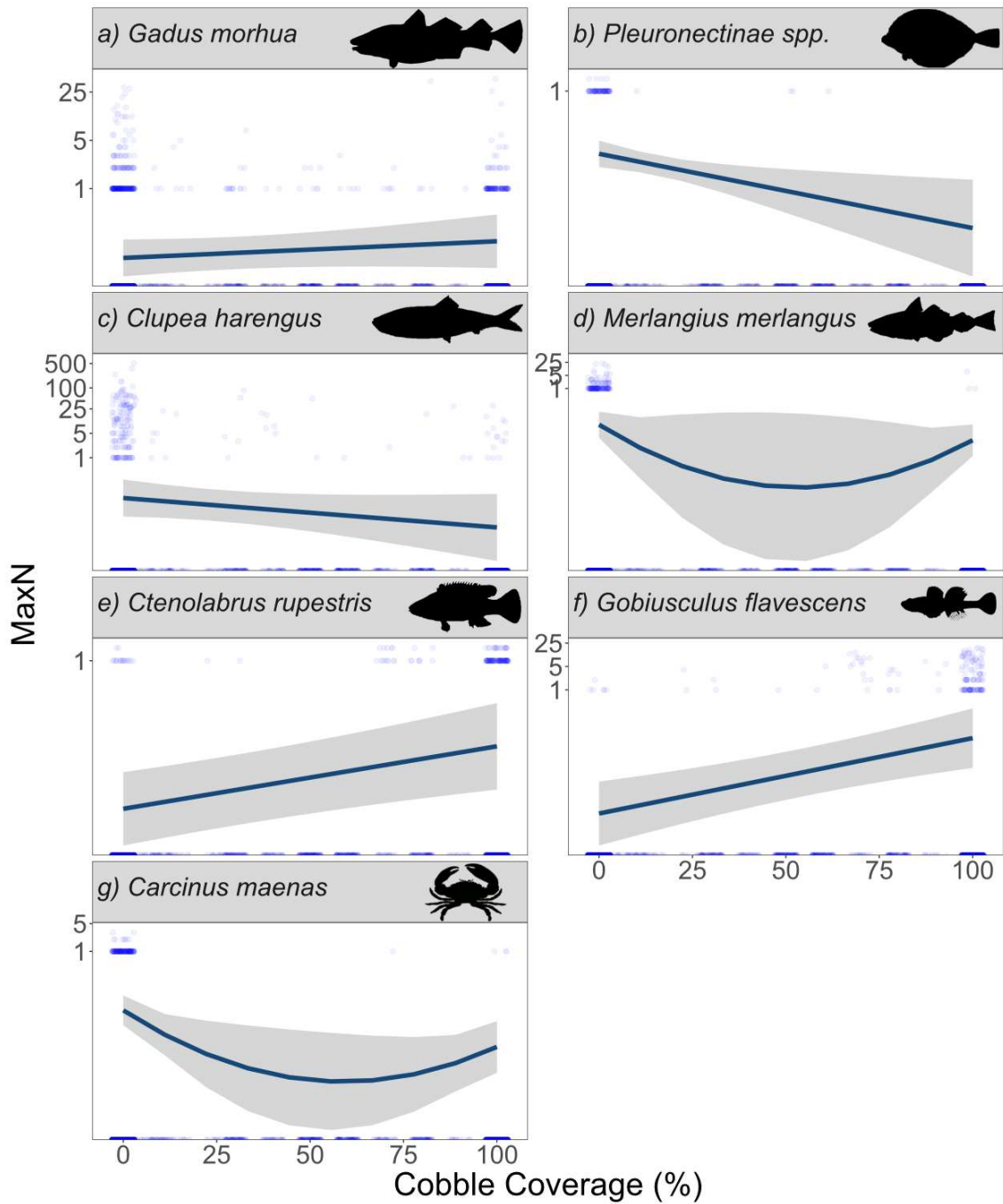
Figure 4.4 – Observed and predicted abundances (MaxN) recorded by UBRUVS across varying cobble coverage. See caption of Fig. 4.3 for explanation of the plots. Results are shown for each of the focal species: (a) Atlantic cod (*Gadus morhua*); (b) Flatfish (*Pleuronectinae* spp.); (c) Atlantic herring (*Clupea harengus*); (d) Whiting (*Merlangius merlangus*); (e) Goldsinny wrasse (*Ctenolabrus rupestris*); (f) Two-spotted goby (*Gobiusculus flavescens*) and (g) Shore crab (*Carcinus maenas*).

by BRUVS whilst UBRUVS failed to demonstrate any significant habitat association for cod. We propose that UBRUVS did not capture individuals hidden within the cobble reefs, whereas hiding individuals were likely attracted to the FOV by the bait and thereby contributed to a stronger species-habitat relationship documented by BRUVS. Previous studies have investigated the demersal habits of juvenile cod; identifying a migratory pattern into shallow soft bottom areas (1-2 m) during the night to feed (Pihl, 1982) and a preference for cobble or rocky habitats in the presence of predators in laboratory studies (Fraser et al., 1996; Gotceitas et al., 1995).

Table 4.3 – Overview of previously documented relationships between species abundance and increasing cobble coverage for the seven focal species of this study.

Species	Common name	Association with increasing cobble coverage	Methodology used	References
Gadus morhua	Atlantic cod	Positive	Stereo BRUVS; Free diving submersible with external video cameras; Tagging individual fish (telemetry)	(Elliott et al., 2017; Gregory and Anderson, 1997; Kristensen et al., 2017)
Pleuronectinae spp.	Flatfish	Negative	Seine nets; Dive transects and netting methods (incl. mono-filament, fyke and gill)	(Jenkins and Wheatley, 1998; Pihl and Wennhage, 2002)
Clupea harengus	Atlantic herring	Negative	Mono-filament nets and stomach analysis; Personal observation	(Stål et al., 2007; Stevenson and Scott, 2005)
Merlangius merlangus	Whiting	Negative	Laboratory study (incl. habitat choice trials); Stereo BRUVS	(Atkinson et al., 2004; Elliott et al., 2017)
Ctenolabrus rupestris	Goldsinny wrasse	Positive	Dive transects and netting methods (incl. mono-filament, fyke and gill); Mono-filament nets and stomach analysis	(Pihl and Wennhage, 2002; Stål et al., 2007)
Gobiusculus flavescens	Two-spotted goby	Positive	Stereo UBRUVS; Dive transects	(Perry et al., 2018; Wilkins and Myers, 1992)

| *Carcinus maenas* | Shore crab | Negative | Personal observation | (Cohen et al., 1995; Grosholz and Ruiz, 1995) |

However, there are fewer studies on adult cod demersal behaviour (Table 4.3; Kristensen et al., 2017). When comparing younger with older juvenile cod, Gregory and Anderson (1997) found older cod associated more with rocky habitats compared to younger cod, which were more abundant near habitats of finer particle size (e.g. gravel). This could imply that as cod age, they associate more with rocky habitats, perhaps because older cod require larger interstitial spaces to accommodate the larger body size. However, the age of the cod recorded in the present study is unknown and future efforts to elucidate their demersal habitat preferences across different life stages are warranted.

Generally, the abundance of two-spotted goby and goldsinny wrasse increased with cobble coverage, although BRUVS revealed a slight decrease in wrasse abundance and a plateauing mean abundance of gobies at high cobble coverage. Previous studies investigating two-spotted goby (Perry et al., 2018; Wilkins and Myers, 1992) and goldsinny wrasse (Pihl and Wennhage, 2002; Stål et al., 2007) have also identified a preference for rocky compared to soft bottom habitats (Table 4.3). Two-spotted goby males are territorial nest holders, typically adopting hard substrates as nesting sites from where they attract females (Utne-Palm et al., 2015). Wrasses play an important role in structuring rocky reef ecosystems by preying on small algae-eating amphipods (Olsen et al., 2019), while serving as prey species for larger marine predators, such as Atlantic cod (Enoksen and Reiss, 2018). Both RUVS methods used in this study confirmed the importance of hard substrate for wrasse and goby. The use of bait did not seem to affect the capability of BRUVS to capture habitat associations for wrasse and goby, as the relationships for both species were clearly defined with significant first and second order terms of cobble coverage in the BRUVS models (Table 4.2). Small individuals often appeared attracted to the overall activity surrounding the bait, even in the presence of larger predators such as cod, suggesting that a potential bias in BRUVS towards sampling dominant large-sized predators may be minimal.

In contrast, abundances of flatfish, whiting and herring decreased as cobble coverage increased. Flatfish often exhibit preference for featureless sand habitats (Table 4.3; Jenkins and Wheatley, 1998; Pihl and Wennhage, 2002), corroborated by the current study. However, there is less literature available on the association between benthic habitat and species abundances of herring and whiting. Herring is a pelagic fish, typically only utilising hard bottom substrate or macrophytes when spawning (Stevenson & Scott, 2005). Therefore, herring spend less time near the seafloor, suggesting they do not possess a strong preference for a particular benthic habitat. This potential lack of habitat preference was confirmed by the two sampling methods in the current study. UBRUVS failed to record any effects of cobble coverage on herring abundance, whilst BRUVS identified a negative association which was not statistically significant (Table 4.2; $p < 0.1$). Overall, herring can be found in a diverse range of habitats, although Stål et al. (2007) found herring to be most common on soft bottoms (Table 4.3), comparable to the trends observed in the present study. Notably, herring appeared unaffected by the presence of bait, with sporadic encounters consisting of large schools

passing the FOV high up in the water column. Although there are reports of cannibalism of adult herring towards larvae (Corten, 2013; Gröger et al., 2010), the use of herring as the bait type in our study may provide an additional explanation for BRUVS recording a relatively weak habitat association for herring compared to the other focal species. Whiting are similar, being found in a range of habitats, with some evidence of a preference for sand over more complex habitats (Table 4.3; Atkinson et al., 2004; Elliott et al., 2017). The present study supports this association of whiting with soft bottom habitats, although UBRUVS recorded very few individuals of whiting and did not document any significant effect of cobble coverage.

Abundance estimates from underwater videos can be affected by the FOV of a camera at a particular site. For example, reduced visibility or rocks obstructing the view may lower species counts obtained from videos. To address these concerns, we included functional visibility and FOV obstruction as covariates in our models. Our data suggest that including measures of visibility could benefit future RUVS studies. Specifically, there was a significant positive effect of visibility on four out of the seven species abundances (flatfish, herring, two-spotted goby and shore crab), indicating that including visibility in the models for these species helped to explain some of the variation in the data. Identifying the importance of including functional visibility in our models suggests that an extension on the current study would be to use a more rigorous approach to obtain accurate functional visibility measurements. Stereo-RUVS use two cameras which can be calibrated to determine functional visibility with high precision, along with the additional advantage of recording more accurate fish length measurements (Cundy et al., 2017; Perry et al., 2018; Whitmarsh et al., 2017). Using the coarser visibility estimates in the current study was sufficient to evaluate any relative variations in visibility between samples. However, the number of significant visibility effects demonstrate the need to include functional visibility in RUVS studies and therefore highlights the importance of using higher accuracy measurements produced by systems such as stereo-RUVS. These improved measurements would also enhance comparability both within and between studies where visibility varies drastically (Espinoza et al., 2014; Whitmarsh et al., 2017).

In conjunction with our results, BRUVS have been used previously to successfully identify species-habitat relationships (Espinoza et al., 2014). Information on how species abundance changes in relation to habitat variables, such as cobble coverage, helps determine the significance of different habitats for individual species. This is important when designing appropriate management strategies, especially those that involve altering the benthic environment or protecting certain areas. Presently, Natura 2000 legislation involves the protection and active restoration of geogenic (i.e. rocky) reefs (Nature Agency, 2016). For example, Støttrup et al. (2017) reported a reef restoration study where 100,000 tonnes of rocks were deposited in the Kattegat Sea to restore previously extracted boulder reefs. Ultimately, it is vital to know what effects such targeted management strategies have on each species, especially in relation to nearby fisheries (both recreational and commercial).

Results from the current study suggest that BRUVS and UBRUVS are both capable of detecting changes in species abundance, but BRUVS may reveal more robust species-habitat relationships by recording a higher number of individuals that are attracted by the bait. However, it remains particularly challenging to quantify the area sampled by BRUVS. Predicting the maximum

distance at which species are attracted to bait requires accurate bait plume models, which thus far have only been developed for deep-sea conditions (Sainte-Marie and Hargrave, 1987). In coastal areas, such as in the present study (6-7 m), water turbulence and temperature are more variable compared to deep sea environments; there is also a greater number of species, each with different foraging strategies and odour detection thresholds (Colton and Swearer, 2010; Hardinge et al., 2013; Priede and Merrett, 1996). Without a suitable model that takes these variables into account, abundance data from BRUVS cannot be converted into absolute density estimates, which could be compared between studies (Harvey et al., 2007; Willis and Babcock, 2000). Still, even though the size of the bait plume was unknown in the present study, the plume did not appear to attract fish from surrounding habitats as we did not observe major deviations from previously documented habitat associations (Table 4.3). It is possible that the use of bait in the BRUVS deployments could have inflated rather than obscured species-habitat relationships, yet the underpinning mechanisms for such a pattern remain unknown and should be examined in future studies.

In conclusion, this study revealed that different RUVS are able to identify previously documented habitat associations for temperate marine species in a patchy coastal area. Thus, this non-extractive technique with minimal benthic impact represents a promising tool for fisheries management. BRUVS documented more robust species-habitat relationships for three out of seven focal species compared to UBRUVS, and identified similar habitat associations for the remaining species. Ultimately, this indicates that equipping RUVS with bait does not obscure relationships between habitat variables and species abundances. BRUVS clearly identified changes in abundance for each species across habitats and provided the additional advantage of higher statistical power over UBRUVS (Bernard and Götz, 2012; Watson et al., 2005). Collectively, these findings support the use of BRUVS as a monitoring tool to address important knowledge gaps in marine ecology.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2020.151315.

Contributors

J.C.S., J.G.S. and B.M.K. conceived the study design; G.R., H.F., B.M.K. and J.C.S. carried out the fieldwork; N.R., J.L.B. and G.R. analysed the video footage; N.R., T.W. and H.B. performed the statistical analysis; N.R. and T.W. led the writing of the manuscript. All authors discussed the results and contributed critically to the final draft of the manuscript.

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Chapter 5

Restoring marine ecosystems: the importance of cobble reefs for species diversity and community composition in a degraded coastal system*

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Abstract

Glacial till deposits (varying in size from small pebbles to large boulders) constitute an important type of marine hard-bottom substrate in the Baltic Sea, yet century-long extraction of this material for the construction of e.g. harbors has degraded large areas of hard-bottom reefs in the region. Cobble reefs (consisting of rocks with diameter between ~ 6 and 25 cm) represent a remarkably understudied type of habitat relative to other reef systems (e.g. boulder or biogenic reefs) and few efforts to date have been carried out to restore this important type of habitat. In this study, we report on the effects of cobble reef restoration on species diversity and the early colonization by marine organisms. Cobble reefs were restored during the winter of 2017-2018 and were constructed as 20 small reef units (truncated pyramids) that alternated in reef height between 0.6 m and 1.3 m, to allow for an investigation into the effect of reef height on the associated marine community. We monitored biodiversity (expressed as Hill numbers) and the relative abundance (expressed as MaxN) of three prominent marine taxa (cod *Gadus morhua*, herring *Clupea harengus* and shore crab *Carcinus maenas*) using baited remote underwater video systems (BRUVS). A comparison was made with multiple target reference sites (i.e. natural reefs) and negative controls (i.e. soft-bottom sites) through a before-after control-impact (BACI) design. Video monitoring revealed an overall decline in diversity and the abundance of focal taxa (except for herring) across control and reference areas from 2017 to 2018, and we subsequently performed BACI ratio computations from Bayesian models to facilitate probabilistic inference on the performance of the restored reefs. Relative to soft-bottom sites, we found high probabilities (> 90%) that restored reefs positively affected Hill-Shannon and Hill-Simpson diversity, but only a moderate probability (74.6%) of having a positive effect on species richness, while probabilities were high for all metrics relative to natural reefs (> 99%). These findings suggest that the declines in species diversity within the study area, whether signifying mere yearly fluctuations or long-term deteriorating trends, were to some degree alleviated at the restoration sites. We found strong evidence of a positive restoration effect on opportunistic shore crab (> 99%), which given their high predation rates on mesograzers could have implications for the development of perennial macroalgae at the restored reefs over time. However, the presence of cobble substrate on the seabed concurrently induced a strong negative correlation of shore crab with cod, its most dominant predator in the WBS, and combined with an 88% probability of a positive restoration effect on cod, these results suggest a promising potential for a top-down control on shore crab numbers at the restored reefs. We did not detect any effect of reef height on the community composition of early colonizing species, yet long-term effects of reef height, given the potential development of perennial macroalgal communities over time, remain unknown and should be further investigated. While the extraction of boulders (diameter > 26 cm) from Danish waters ceased in 2010 after a ban was introduced, rocks of smaller size are not covered by the ban and are currently still removed during other extractive activities (e.g. mussel dredging). This study demonstrates that cobble reefs constitute an important type of coastal habitat that benefits marine biodiversity, hosts a unique assemblage of reef taxa and drives a large number of species correlations

within the WBS. Our findings call for improved management actions aimed at conserving cobble habitats and will hopefully serve as a motivation for similar initiatives to restore cobble reefs in the near future.

5.1. Introduction

Marine habitats are under continued pressure from a wide range of anthropogenic impacts including pollution, urbanization, resource extraction and climate change (Lotze et al., 2006; Andersson et al., 2015; Vince & Hardesty, 2017; Lin & Yu, 2018; McDermott, Meng, McDonald, & Costello, 2019). The degradation of coastal areas is often a relatively slow process, for example when coastal development alters the environment over human generations (Sundblad & Bergström, 2014) or in case of eutrophication leading to the gradual shift from seagrass meadows to faster growing algal species in shallow bays (Waycott et al., 2009). However, degradation can also take place on much shorter time scales, for example by mass bleaching events triggering rapid transformations of reef topography (Hughes et al., 2018), and may even occur instantaneously after destructive overexploitation of biogenic (e.g. oyster) reefs (Beck et al., 2011) or targeted excavation of hard substrates (Dahl, Lundsteen, & Helmig, 2003). Geogenic (i.e. rocky) reefs often constitute an oasis among vast areas of soft bottom by providing hard surfaces for the attachment of macroalgae and settlement of sessile organisms, and thereby can host a rich assemblage of benthic and demersal species (Støttrup et al., 2014; Coolen et al., 2015; Brzana & Janas, 2016). The permanent removal of hard substrates from the seabed is therefore particularly detrimental to these communities, as the associated alterations in seabed topography and structural complexity are deemed irreversible without human intervention (i.e. restoration efforts; Støttrup et al., 2017).

For more than a century, large amounts of marine rocks were extracted in Danish coastal areas for the construction of piers, jetties and other coastal structures (Støttrup et al., 2014; Kristensen et al., 2017). While extraction of boulders has been prohibited since 2010 (Kristensen et al., 2017), it is still legal to extract other substrates, including gravel, pebble and cobble in dedicated marine areas. Similar to boulder areas (Kristensen et al., 2017; Liversage et al., 2017; Liversage & Chapman, 2018), areas with smaller rocks may also support diverse marine life, particularly juvenile eel (*Anguilla anguilla*) and Atlantic cod (*Gadus morhua*) (Lough et al., 1989; Tupper & Boutilier, 1995; Christoffersen et al., 2018). Concurrently, cobble areas may also provide elevated post settlement survival and recruitment success compared to sand bottom areas (Tupper & Boutilier, 1997) and facilitate spawning of commercially valuable fish species. Both in the North Sea and in the Baltic Sea, Atlantic herring (*Clupea harengus*) often spawn in areas with rocks of variable sizes, either directly onto the hard surfaces or on vegetation growing on the rocks, including various macroalgae species (Groot, 1980; Aneer et al., 1983; Geffen, 2009; Kanstinger et al., 2018). Local herring populations may diminish if historic spawning areas become

unavailable (Groot, 1980; Wolff, 2000), highlighting the importance of hard substrate availability for this commercial species. Spawning of herring occurs mainly in coastal regions and is restricted to water depths less than 10 m in the Baltic Sea (Aneer, 1989). Herring depositing eggs may attract various predators that feed directly on the herring or on the eggs that are attached to the benthic surfaces (Kotterba et al., 2014). For example, three-spine stickleback, (*Gasterosteus aculeatus*) can consume large quantities of herring eggs (Kotterba et al., 2017). Therefore, herring spawning activities could induce high abundances of forage species (e.g. three-spine stickleback) that may in turn provide food resources for larger fish, potentially leading to trophic interactions.

Ecological restoration, i.e. the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed (Clewell, Aronson, & Winterhalder, 2004), is increasingly being applied in a global effort to counteract habitat loss. However, marine habitat restoration is still an emerging field relative to terrestrial restoration (Abelson et al., 2016; Hale et al., 2019) and the success of restoration efforts is significantly challenged by the inherently dynamic conditions of the marine environment (Bayraktarov et al., 2015). The majority of restoration efforts within marine systems involves the revegetation of native marine plants or the removal of invasive plant species (Hale et al., 2019), while reef restoration constitutes a smaller proportion and has been mainly limited to biogenic reef (i.e. coral and shellfish reefs) restoration (Gilby et al., 2018; Williams et al., 2019). Efforts to restore boulder and cobble reef habitats are scarce, yet the few empirical studies that carried out post-restoration monitoring have shown promising results (Kilfoyle et al., 2013; Støttrup et al., 2014; Wilms et al., 2021). For example, more than 100,000 tons of previously extracted marine boulders were recently restored in the Kattegat Sea between Denmark and Sweden (Støttrup et al., 2017). The restoration efforts resulted in higher abundance (Støttrup et al., 2014) and longer residence time (Mikkelsen et al., 2013; Kristensen et al., 2017) of commercially important species and apex predators at the reef compared to pre-restoration sites. In addition, restoration of boulder reefs has recently been advocated as a foundation for restoring shellfish reefs, by which the combined benefit of 'multi-habitat restoration' could potentially outweigh the benefit of restoring either of the habitat types in isolation (Liversage, 2020). Still, the apparent scarcity of temperate reef restoration efforts implies that knowledge on effective restoration strategies for this important habitat type remains limited (Støttrup et al., 2017). One important reef feature that is easily manipulated during restoration efforts is reef height, which could potentially yield important consequences in terms of both restoration costs and efficacy. For example, constructing coastal reefs to protrude higher up the water column might facilitate rapid algal growth due to increased light intensities. However, the construction of high reefs implies substantial increases in restoration costs because more source material is required relative to the seabed footprint of the reef. Empirical evidence is therefore needed to identify potential effects of reef height and guide restoration managers to most effectively allocate their limited resources. While reef height has been identified as an important factor driving restoration success for oyster reefs (Lipcius et al., 2015; Colden, Latour, & Lipcius, 2017), the effect of reef height on fish and invertebrate communities associated with cobble reefs remains poorly understood.

In this study, we report the early effects of cobble reef restoration on the fish and macroinvertebrate community in a coastal area of the Western Baltic Sea (WBS), where decade-long extraction of hard substrates and coastal eutrophication have severely degraded the marine ecosystem. We monitored the diversity and relative abundance of inhabitant species using baited remote underwater video systems (BRUVS), a non-invasive sampling technique that is particularly useful for sampling marine communities in vulnerable areas, e.g. within protected areas or restoration sites (Cappo et al., 2003; Espinoza et al., 2020). We employed a BACI sampling design, by monitoring control and impact (i.e. restoration) sites before and after the reef restoration and assess how relative differences in response variables varied over time. Restored reefs were compared with sand sites and natural reefs in the study area (i.e. negative control and target reference, respectively; Wortley, Hero, & Howes, 2013), to investigate how the newly restored reefs developed relative to both these types of reference sites. We hypothesize that the restoration of cobble reefs positively affected marine biodiversity, as well as the abundance of cod, eel and herring by providing hard substrate for herring to spawn and suitable habitat (i.e. increased shelter availability) for young cod and eel. Finally, we expected the community composition of the restored reefs to deviate from sand controls and become more similar to natural reefs in the study area, with variations in reef height inducing further distinction within the restored reef assemblage.

5.2. Materials and Methods

5.2.1. Study area

We conducted our study in Sønderborg Bay, which is a shallow coastal area within the Flensborg Fjord located between Denmark and Germany (Fig. 5.1). Across the bay, large areas of soft sediment (mostly sand and muddy sand) locally form patchy mosaics with naturally occurring hard substrate (ranging in size from small pebbles to boulders). The bay has been subjected to long-term extraction of cobbles and boulders, yet current Natura 2000 legislation has halted further extractive activities of large rocks from the Danish seabed (Kristensen et al., 2017). Six field sites were monitored during the study (Fig. 5.1), including two soft sediment sites designated as controls (Kegnæs Ende and Viemose), two natural cobble reefs serving as reference sites (Spar Es and Vesterhage) and two experimental sites (Hvide Mur and Stenholt). The experimental sites consisted of empty sand bottoms before the reef restoration, similar to control sites (soft sediment), but hosted multiple small reef units after restoration efforts were complete. Sampling took place between 6 m and 7 m of water depth along the coastline, within designated areas that had a maximum size of 500 m in length and 200 m in width (polygons in Fig. 5.1).
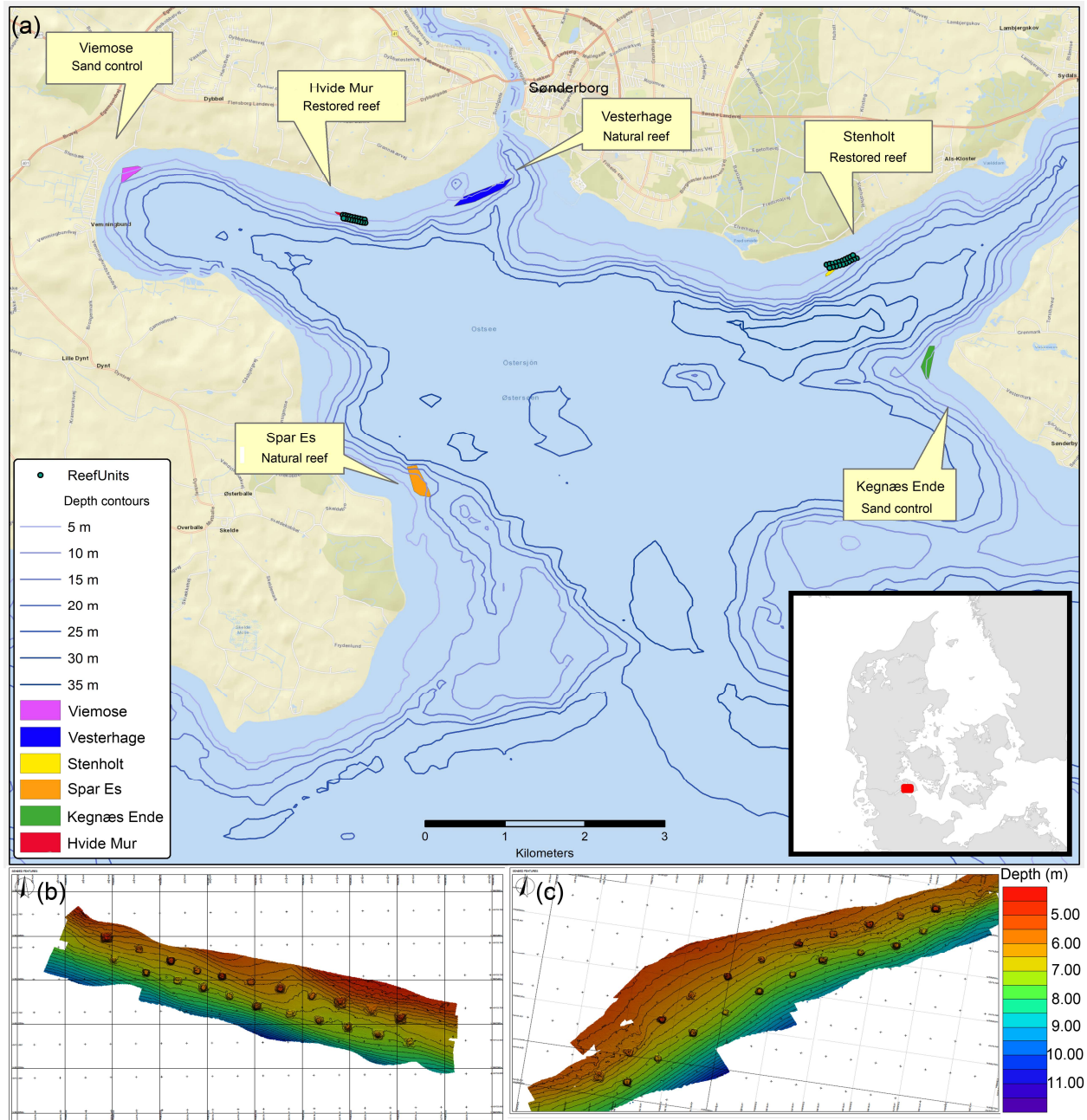
Figure 5.1 – Location and spatial configuration of the restored cobble reefs. This study was conducted in Sønderborg Bay within Flensborg Fjord (a). Six field sites were monitored before and after the reef restoration, including four references sites (polygons) and two restoration sites (small circles). Reference sites consisted of two sand controls (Kegnæs Ende and Viemose) and two natural reefs (Spar Es and Vesterhage). The cobble reefs were restored in 10 x 2 small reef units shaped as truncated pyramids at Hvide Mur (b) and Stenholt (c), and alternated in height between low (0.6 m) and high (1.3 m) reef units. Map credits: Jeppe Olsen.

5.2.2. Reef design and deployment

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Cobble reefs were constructed over two months during the winter of 2017-2018. Deployments were carried out from an anchored ship using machinery that extended from the ship to the seabed to ensure that the individual reef units were created in agreement with the planned designs. Reef units were deployed in two lines situated at water depths of 6 m and 7 m (one line of reef units at each water depth). In each location (Hvide Mur and Stenholt; Fig. 5.1), 20 reef units were divided into 10 reef units placed on the 6 m line and 10 reef units placed on the 7 m line. Along these two lines, reef units alternated between low and high reef units (i.e. 0.6 m or 1.3 m high). Reef shapes and locations were confirmed by SCUBA diving shortly after deployment. SCUBA diving also revealed that rocks with a diameter up to approximately 40 cm were occasionally included in the reefs. This meant that the average rock size for some reef units was larger than cobble and approached 30 cm.

5.2.3. Data collection

Field sites were monitored in April and May of 2017 and 2018 using baited remote underwater video systems (BRUVS). Remote video sampling is a non-invasive monitoring technique, with minimal impact to the benthic environment (Cappo, Harvey, Malcolm, & Speare, 2003). The RUVS setup consisted of a metal pole (3 cm in diameter; 100 cm in height) fixed to a concrete base (measuring 45x45x5 cm in length, width and height respectively) for stable positioning on the seabed. Cameras were positioned horizontally to the pole at a height of 20 cm above the seafloor. We attached an 80 cm long bait arm below the cameras, holding a mesh bait bag containing 500 g of fresh Atlantic herring (*Clupea harengus*) chopped into 1-2 cm pieces. Increments of 10 cm were marked along the bait arm for use as a reference for visibility estimates. We used GoPro cameras (Hero 3, 3+ & 4, www.gopro.com) at 720p video resolution and 30 frames per second (NTSC) for recording. BRUVS started recording immediately upon deployment and ran continuously to capture the effect of the bait plume. We equipped BRUVS with an extra battery (BacPac; www.go-pro.com) to ensure a minimum soak time of 1 hour, and up to a maximum of 2.5 hours, which is in agreement with recommendations on appropriate soak times for effectively comparing fish assemblage patterns (Harasti et al., 2015). No artificial light sources were used in the present study and BRUVS were deployed at least two hours before sunset to ensure adequate light conditions. Day length varied between 13 and 17 hours during the study period. Deployment of the BRUVS resembled procedures used in previous studies (see Langlois et al., 2018 for a detailed description). We used a side scanner (Lowrance Elite-7 Ti; www.lowrance.com) to identify the exact locations of the individual reef units at restored sites, as well as to verify suitable sandy bottoms and hard substrates within control and natural reef sites, respectively. At each of the sites, we kept to a maximum of two temporally overlapping deployments and actively maximized the distance between the cameras to minimize the risk of spatial overlap of the bait plumes.

5.2.4. Video analysis

Recordings were analyzed in VLC media player (VideoLan; www.videolan.org) by video observers. The MaxN metric, i.e. the maximum number of individuals of a particular species within a single video frame (Ellis & DeMartini, 1995; Willis & Babcock, 2000; Cappo et al., 2003), was used as a measure of relative abundance in this study. The use of MaxN prevents duplicate counts of individuals within a video sample and is widely regarded as a conservative estimate of species abundance. Species in the recordings were identified to the lowest possible taxonomic level with the use of compiled reference images. In case identification to species level was not feasible, individuals were grouped into higher taxonomic categories, e.g. by genus or family. For example, flatfish species can be particularly challenging to identify to species level due to their coloration resembling the seabed, while sand gobies were often too small or distant from the camera to identify. Accordingly, flatfishes that could not be identified to species-level were grouped as either left-eyed family (*Scophthalmidae* sp.), right-eyed family (*Pleuronectidae* sp.) or on order level (*Pleuronectiformes* sp.) in case the position of the eyes was not visible. Sand gobies for which species ID could not be obtained were grouped by genus (as *Pomatoschistus* sp.) to keep them separate from the often distinctly larger black goby (*Gobius niger*). Ultimately, for each sampling unit, data were collected on species identification, MaxN count, cobble coverage (%), vegetation coverage (%) and functional visibility (m). We used the tape markers on the bait arms to designate individuals to size classes with 5 cm increments, i.e. 0-5 cm, 5-10 cm etc. In rare occasions, an individual of a conspicuously different size (i.e. two or more size classes larger or smaller than the MaxN group) was observed outside the MaxN frame, in which case we included the individual in the total MaxN count for that species (similar to Watson et al., 2010). Cobble coverage was defined as the percentage of visible seafloor comprised of cobble stones, while the vegetation coverage was assessed by estimating the percentage of visible substrate covered by macroalgae. We defined functional visibility as the furthest distance from the camera at which species were still identifiable, which depended highly on water turbidity and was estimated using reference images and the tape markers in front of the cameras.

5.2.5. Statistical analysis

We explored three different taxonomic diversity indices to assess the effect of reef restoration on marine biodiversity in the study area. Specifically, we assessed three commonly used integer orders (q) of diversity from the continuum of Hill numbers (Hill, 1973; Jost, 2006), defined as:

$$^{q}D = \left(\sum_{i=1}^{S} p_i^{q} \right)^{1/(1-q)} \tag{1}$$

with D denoting the diversity, S the total number of species in the sample, p_i the proportion of individuals in the sample belonging to species i and q representing the diversity order. Thus, for the first integer order ($q = 0$), the Hill diversity is computed by summing all the unique species encounters (i.e. species

richness) for each sample. The second integer order ($q = 1$) is undefined in Eq. (1) and therefore its limit is used:

$$^1D = e^{-\sum_{i=1}^{S}(p_i \ln(p_i))} \tag{2}$$

which we hereby refer to as the Hill-Shannon diversity index (Roswell, Dushoff, & Winfree, 2021). The final integer order ($q = 2$) is equivalent to the inverse of the widely used Simpson index:

$$^2D = \frac{1}{\sum_{i=1}^{S}(p_i)^2} \tag{3}$$

and is hereby referred to as the Hill-Simpson diversity index (Roswell et al., 2021). These three Hill diversity indices differ in their sensitivity to common and rare species, with Richness ($q = 0$) completely ignoring relative abundance (and therefore being very sensitive to rare species), Hill-Shannon ($q = 1$) putting equal emphasis on common and rare species, and Hill-Simpson ($q = 2$) emphasizing common species (Jost, 2006; Roswell et al., 2021).

We fitted Bayesian hierarchical models to obtain probabilistic inference on the effect of cobble reef restoration on the three taxonomic diversity indices described above, in addition to the effect on the relative abundance of three prominent focal species in this study. Specifically, we modelled the univariate responses through Generalized Linear Multilevel Models (GLMLMs), which all included the factors Year (two levels: pre- and post-restoration), Treatment (three levels: Control, Natural reef, Restored reef) and their interaction. We then focused on the interaction to compare restoration sites with reference sites, before and after the reef restoration (i.e. a BACI comparison; Underwood, 1991). Additional covariates were included in all models to account for inherent variations in BRUVS recording time (i.e. soak time) and functional visibility, which was achieved by adding the natural logarithm of these two covariates and effectively express them as exposure terms (McElreath, 2016; Zuur & Ieno, 2016). We included varying intercepts per Site ID (Fig. 5.1), to incorporate inherent correlations between BRUVS recordings from the same field site due to similar local conditions. In addition, this structure provides the flexibility to account for varying sampling efforts per field site (Table S5.1), by facilitating partial pooling (i.e. information sharing) across the grouping levels to estimate the population of varying intercepts in the lowest model hierarchy (Nalborczyk, Batailler, Loevenbruck, Vilain, & Bürkner, 2019). All response variables were initially modelled using a Poisson distribution with a log-link, after which posterior predictive checks were performed to assess the distributional fit (Jonah Gabry, Simpson, Vehtari, Betancourt, & Gelman, 2019). The species richness model showed severe underdispersion, i.e. the variation in the data was less than expected from simulated datasets. We addressed this by fitting a Conway-Maxwell Poisson (CMP) instead (Huang, 2017), a distribution that is increasingly being used in ecological studies to accommodate

for underdispersion (Brooks et al., 2019). The remaining two Hill diversity metrics were modelled using a Gamma distribution, as these two responses had a non-negative continuous format, while the relative abundances of three focal species followed a negative binomial (NB) distribution due to overdispersion under the regular Poisson (all models used the log-link). All models were run on four Markov chains with a total of 20,000 iterations (10,000 discarded as warm-up). We assigned $N(0,3)$ priors on all population-level effects, half-Cauchy (location: 0, scale: 2.5) on the shape parameter of the CMP and NB (due to its flat-tailed distribution allowing for very large shape values) and an Exponential (rate: 1) prior on the standard deviation of the varying intercepts (McElreath, 2016). Prior predictive checks were performed to verify the use of appropriate priors for all model parameters (Gabry et al., 2019). Model convergence was assessed by inspecting chain mixing and by verifying that the potential scale reduction statistic was equal to one for all model parameters (Gelman & Rubin, 1992).

Next, to obtain probabilistic inference on the effect of reef restoration, we computed BACI ratios via Markov Chain Monte Carlo (MCMC) sampling following the protocol described in Conner et al. (2016) and the equations from Wilms et al (2021). Briefly, "before" ratios are calculated for each MCMC iteration by extracting the fitted values (on the response scale) for impact (i.e. restoration) and control treatments before the impact event (Time: pre-restoration) and then dividing them. This calculation is then repeated for fitted values after the impact event (here Time: post-restoration). The "after" ratios are then divided by the "before" ratios to obtain a measure of proportional change between restored reefs and control sites over time, i.e. the BACI ratio. Hence, we obtain a BACI ratio for every post-warmup iteration in the Bayesian model (in our case 40,000 BACI ratios) allowing for probabilistic interpretation by computing the area under the BACI ratio density curve corresponding to any effect size of interest. Since we are interested in the probability of a positive reef restoration effect, relative to reference sites, we compute the area curve corresponding to BACI ratios > 1 to obtain the probability associated with a positive effect. Here, we investigated the effects of reef restoration both in relation to sand control sites and to natural reef sites, to assess how the restored reefs developed relative to both these types of reference sites. Further details on computation and interpretation of the BACI ratios can be found in Wilms et al. (2021).

Finally, we examined the effect of reef restoration and reef height on the community composition using a Bayesian ordination and multivariate regression analysis. This approach allows for running a model-based unconstrained ordination by regressing species against unknown (latent) variables, i.e. a pure latent variable model (LVM; Warton et al., 2015; Hui, 2016), and thereby facilitates model comparison through information criteria and validation of distributional assumptions. To assess potential effects of reef height on the community composition, we filtered the data on Year (post-restoration) and Treatment (restored reefs) to examine if the assemblage at restored reefs were to any extent shaped by the height of the reef units (Fig. 5.1). Both the "restoration LVM" and the "reef height LVM" followed a negative binomial distribution as this substantially improved the fit compared with the initial Poisson (in terms of information criteria and model assumptions). We defined "random" site-level row effects to

incorporate correlations in recordings within the same site, similar to the univariate analysis part, and excluded species occurring in less than three BRUVS deployments. We subsequently investigated the importance of cobble coverage in shaping correlations among species in the study area, by running a correlated response model (CRM) to disentangle species correlations explained by variations in cobble coverage from residual ordinations unexplained by this covariate (Warton et al., 2015).

All analyses were run in R version 3.6.3 (R Core Team, 2020). We used the package *brms* (Bürkner, 2017) to fit the Bayesian hierarchical models through the R interface of the STAN programming language (Carpenter et al., 2017). Model comparisons were performed with *loo* package version 2.3.1 (Vehtari et al., 2020) and we visualized the posterior predictive checks with *bayesplot* version 1.7.1 (Gabry & Mahr, 2019). The LVMs were run with package *boral* version 1.8 (Hui, 2016).

5.3. Results

5.3.1. Sampling effort and observed marine community

Field sampling resulted in a total of 379 BRUVS deployments across the study period, with 134 cameras deployed during pre-restoration (Year 2017) and 245 cameras during post-restoration (Year 2018) sampling. The average soak time per camera amounted to 101 (SD: 16.6) minutes. We recorded 44 unique marine species across the two years, of which 29 were observed in 2017 and 41 in 2018. At the reef restoration sites (Fig. 5.1), we observed 15 species before restoring the reefs and 31 species after the restoration. Atlantic herring (*Clupea harengus*) was the most abundant species in terms of the number of individuals recorded (9048 individuals), yet herring ranked 5th in terms of frequency of occurrence reflecting the sporadic nature in which we observed large herring schools. The second and third most abundant species observed were Atlantic cod (*Gadus morhua*) and sand eel (*Ammodytidae* sp.), respectively, while cod, shore crab (*Carcinus maenas*) and common starfish (*Asterias rubens*) were the top three species in terms of encounter frequency. A complete overview of the observed species list, including summaries of relative abundance and encounter frequencies, is provided in Table S5.1. BRUVS recordings indicated large variations in functional visibility between the two sampling years, with a mean visibility of 1.1 m (SD: 0.2 m) in 2017 compared with 2.3 m (SD: 0.4 m) in 2018.
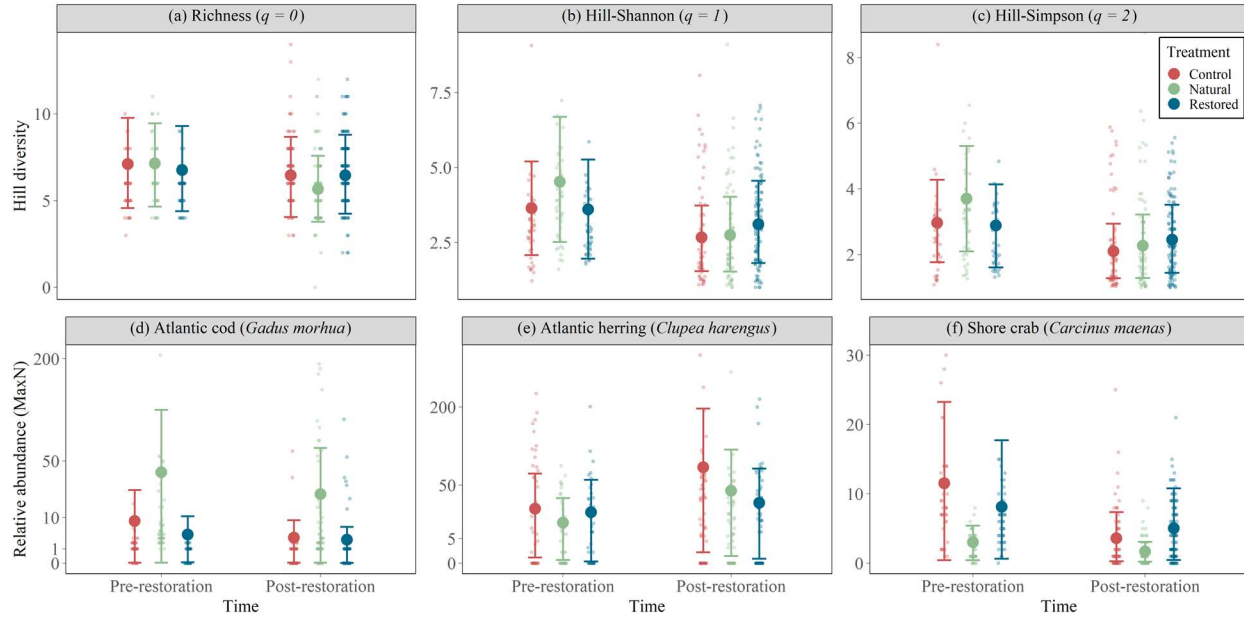
Figure 5.2 – Species diversity and relative abundance of three focal taxa documented during pre- and post-restoration monitoring. The posterior means and 95% credible intervals (CI) are denoted by the large enclosed circles and whiskers, respectively. Raw observations are indicated by the small transparent dots that were jittered horizontally to avoid excessive overlap. Note that some y-axes were root transformed to allow for combined visualization of model output and raw data points. Results are presented for species richness (a), Hill-Shannon diversity (b), Hill-Simpson diversity (c) and the relative abundance (MaxN) of Atlantic cod (d), Atlantic herring (e) and shore crab (f).

5.3.2. Diversity metrics and focal species responses

Our univariate analyses revealed that species diversity, based on the three Hill diversity components examined, exhibited a generally decreasing trend across the bay between the two sampling years (Fig. 5.2a-c). Richness (i.e. the number of unique species observed per camera deployment) decreased on average by 8.7% at sand reference sites and by 20.4% at natural reef sites (Fig. 5.2a). Using our BACI design to compare restoration and reference sites through time, we found only a moderate probability of 74.6% that richness increased as a result of reef restoration, relative to sand controls (Fig. 5.3a; q = 0). However, relative to natural reefs, there was a 99.3% probability that reef restoration resulted in higher richness over time (Fig. 5.3b; q = 0). Hill-Shannon diversity similarly showed a decreasing trend at reference sites across the years, with an average 25.9% and 38.9% decrease in diversity at sand controls and reef reference sites, respectively (Fig. 5.2b). Our BACI comparison however indicated high probabilities of a positive restoration effect on Hill-Shannon diversity (Fig. 5.3; q = 1), 91.7% relative to sand controls and 99.9% relative to natural reef sites. These high probabilities are reflective of the relatively stable Hill-Shannon diversity at restoration sites (Fig. 5.2b), relative to the sharp

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declines at both types of reference sites. The final diversity metric, Hill-Simpson diversity, showed very similar trends to Hill-Shannon with declines of 28.4% and 38.1% at sand controls and natural reefs, respectively, across the two sampling years (Fig. 5.2c). Yet, relatively stable Hill-Simpson diversity at restoration sites resulted in a 93.5% probability of a positive restoration effect, relative to sand controls, and a 99.6% probability relative to natural reefs (Fig. 5.3; q = 2).
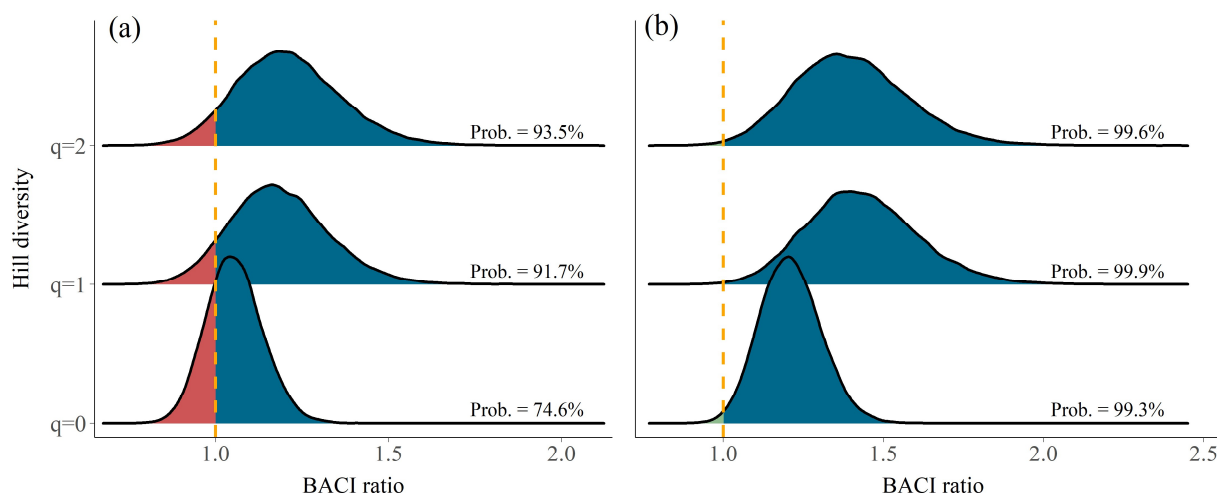
Figure 5.3 – Density curves of BACI ratios computed according to equations from Wilms et al. (2021). BACI ratios were computed for the three Hill diversity metrics used in this study: Richness (q = 0), Hill Shannon (q = 1) and Hill Simpson (q= 2). A BACI ratio of 1 corresponds to equal response variables before and after the restoration (orange dashed line), and the probability of a positive restoration effect (denoted at the base of each curve) is therefore equivalent to the area under the BACI curve for ratios > 1 (blue shaded areas). Restored reefs were compared with sand controls (a) and natural reefs (b) as reference sites.

The relative abundance (MaxN per deployment) of the three focal species also showed strong variations between the two sampling years (Fig. 5.2d-f). Specifically, Atlantic cod decreased on average 55.5% in relative abundance at sand controls while an average decrease of 35.5% was observed at natural reefs (Fig. 5.2d). Declines in cod abundance were less severe at restoration sites across time (18% average decrease), yielding a posterior probability of 88.2% that restoration efforts promoted cod abundance relative to sand control sites (Fig. 5.4a; top panel). While average declines were almost twice as severe at natural relative to restored reefs, incorporating the uncertainty of this estimate through computation of the BACI ratios indicated similar trends between the treatments, with a moderate probability (63.2%) that restored reefs promoted cod abundance relative to natural reefs (Fig. 5.4b; top panel). In contrast, Atlantic herring strongly increased in relative abundance at both the sand controls

Figure 5.4 – Density curves of BACI ratios corresponding to the focal taxa investigated in this study. Details on interpretation of the plots and ratios are provided in the caption of Figure 5.3. Focal taxa included Atlantic cod (*Gadus morhua*; top panel), Atlantic herring (*Clupea harengus*; middle panel) and shore crab (*Carcinus maenas*; bottom panel). Restored reefs were compared with sand controls (a) and natural reefs (b) as reference sites.
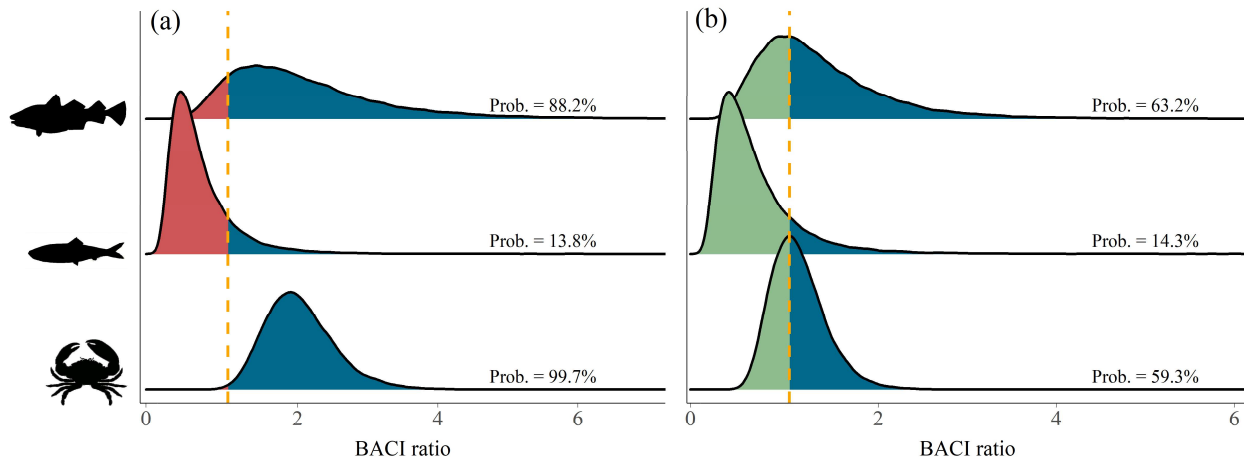
(347% average increase) and natural reefs (306% average increase; Fig 5.2e). Although we did observe an average increase of 99% in herring abundance at restored reefs, this was substantially less compared with the reference sites which therefore yielded low probabilities of a positive restoration effect (13.8% and 14.3% relative to sand controls and natural reefs, respectively; Fig. 5.4; middle panel). Finally, shore crab declined in relative abundance for all treatments during the study period (Fig. 5.2f). The average decline was most severe at sand controls (66.7% decrease), while the decline was similar at natural and restored reefs (38.5% and 34.5% decrease, respectively). These trends were reflected in the BACI ratio distributions (Fig. 5.4; lower panel), with a very high posterior probability (99.7%) of a positive restoration effect on crab abundance relative to sand controls, but a moderate probability (59.3%) relative to natural reefs indicating similar trends in crab relative abundance over time.

5.3.3. Multivariate community assessment

The unconstrained ordination of the species community based on pure latent variable modelling revealed distinct patterns between the various time-treatment pairs (Fig. 5.5a). Most notably, while restoration sites and sand controls had similar assemblages during pre-restoration monitoring, as evidenced by the overlapping confidence ellipses, these assemblages diverged following the reef restoration. A negative LV2 coefficient for the post-restoration assemblages (i.e. downward shift along the y-axis in Fig. 5.5a) indicated substantial inter-annual variation in community composition at sand controls and restoration sites. Concurrently, the positive LV1 coefficient at restored reefs (i.e. shift to the right

along the x-axis in Fig. 5.5a) suggests that the species composition converged more towards the natural reefs. Species associated with pre-restoration sand controls and restoration sites included whiting (*Merlangius merlangus*), brill (*Scophthalmus rhombus*) and sea snails (*Caenogastropoda sp.*). The deviation observed during post-restoration sampling was mainly triggered by garfish (*Belone belone*), flounder (*Platichthys flesus*) and sand goby (*Pomatoschistus sp.*) which were all highly associated with
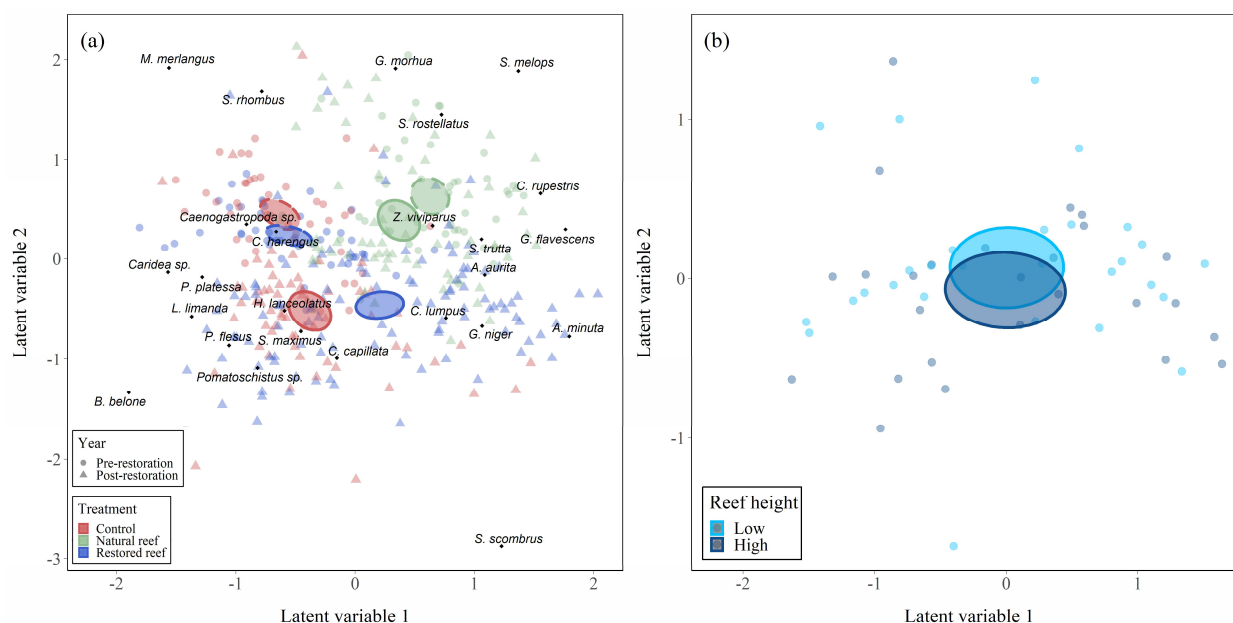
Figure 5.5 – Unconstrained ordinations based on latent variable models (LVMs). The species composition for each BRUVS deployment (small transparent symbols) are regressed against a set of unknown parameters (the latent variables) to visualize community patterns in a low-dimensional plot. Ellipses represent the 95% confidence interval for pre-restoration (dashed ellipses) and post-restoration (solid ellipses) centroids. LVMs were run on all pooled data to assess restoration effects (a) and on restored reefs only to examine potential effects of reef height (b). Positions of the 25 most important indicator species are superimposed as small black dots (based on their latent variable coefficients) to visualize their association with the different time-treatment combinations.

sand controls, whereas transparent goby (*Aphia minuta*), black goby (*Gobius niger*) and lumpfish (*Cyclopterus lumpus)* were associated with restored reefs. The communities at natural reefs as recorded during pre- and post-restoration sampling were very similar, yet a deviation between confidence ellipses is still apparent (Fig. 5.5a) suggesting a small amount of inter-annual variation in composition (although with lower variation relative to sand controls and restored reefs). Here, indicator species for natural reefs included Atlantic cod (*Gadus morhua*), corkwing wrasse (*Symphodus melops*) and goldsinny wrasse (*Ctenolabrus rupestris*). Finally, running a separate LVM on the restored reefs provided no evidence of community differences induced by variations in reef height (Fig. 5.5b).
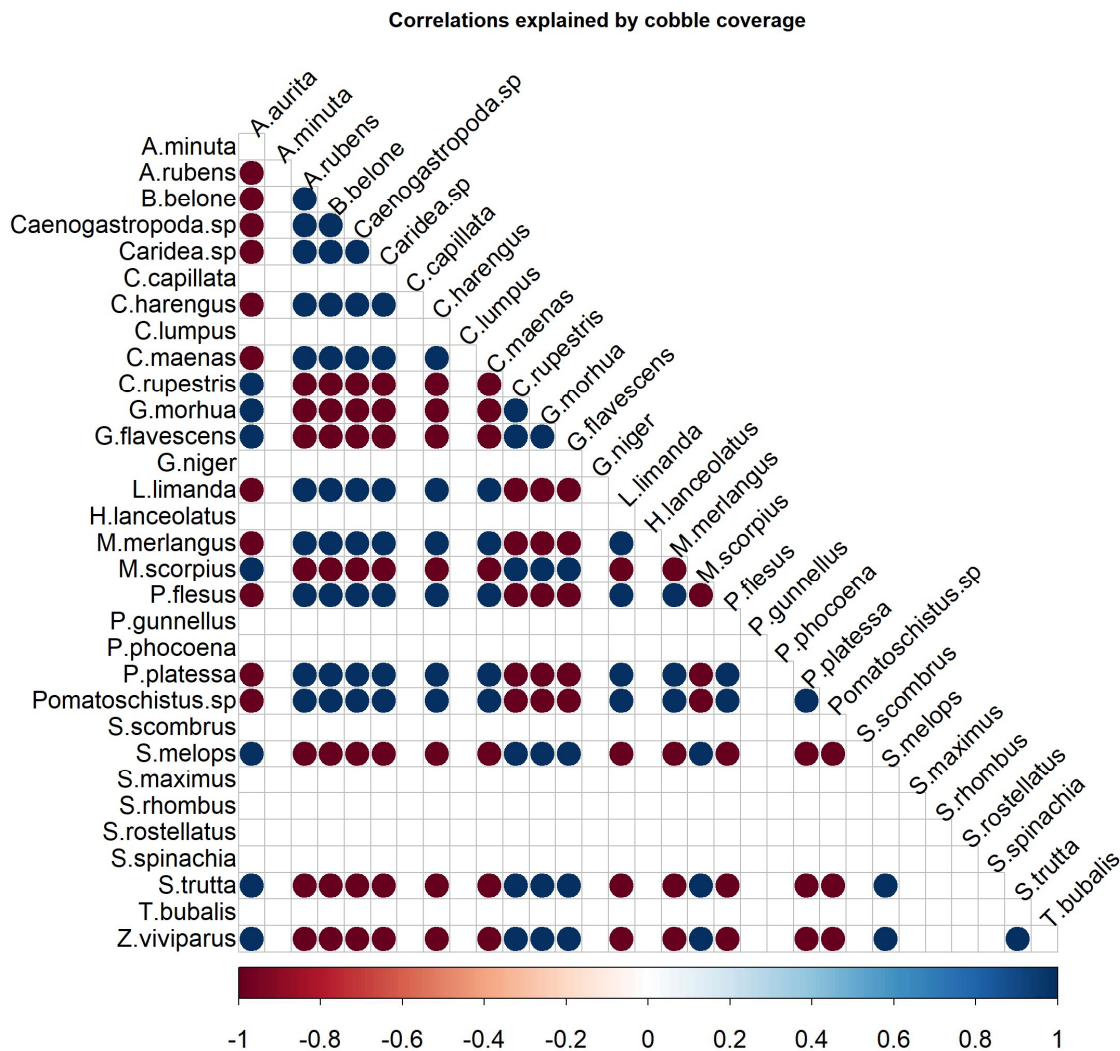
Figure 5.6 – Species correlations induced by cobble coverage on the seabed. The correlation plot is based on a correlated response model (CRM), in which cobble coverage on the seabed (estimated by video observers) is included as an environmental covariate alongside the latent variables that account for residual correlations (Hui_16). Only significant species correlations are presented, based on the 95% CI excluding zero, with the size and color of the circles denoting the strength and sign of the correlations, respectively. For example, cobble coverage explained significant positive associations between Atlantic cod (G. morhua), and e.g. goldsinny wrasse (C. rupestris) or two-spotted goby (G. flavescens), implying that the co-occurrence (or co-absence) of cod and these species was (at least partly, depending on the residual correlations) explained by variations in cobble coverage. In other words, Atlantic cod, goldsinny wrasse and two-spotted goby exhibited similar responses to varying cobble coverage on the seabed, which induced strong positive correlations in their abundances as recorded by BRUVS.

Accounting for reef coverage on the seabed (here cobble coverage) in a correlated response model (CRM) allowed us to disentangle correlations among species induced by the presence (or absence) of cobbles from correlations induced by other environmental factors (i.e. the residual correlations). Results from the CRM revealed strong intra-specific correlations (either highly positive or negative) due to cobble coverage for many of the observed species (Fig. 5.5). For example, cobble coverage induced strong positive correlations between Atlantic cod (*G. morhua*) and a number of reef-associated taxa including goldsinny wrasse (*C. rupestris*), two-spotted goby (*G. flavescens*), shorthorn sculpin (*M. scorpius*) and eelpout (*Z. viviparus*). On the other hand, low coverage of cobble on the seabed explained strong positive correlations between benthic predators (*P. flesus* and *P. platessa*) and other soft-bottom taxa such as whiting (*M. merlangus*) and sand goby (*Pomatoschistus sp.*). The residual correlations plot indicated a substantial number of species correlations unexplained after accounting for cobble coverage, which could for example be due to other environmental factors (e.g. water temperature or salinity) or biotic (e.g. predator-prey) interactions. A notable example includes the greater sand eel (*H. lanceolatus*), which did not show any significant correlations with other species due to cobble coverage but a large number of significant residual correlations (Fig. 5.5; Fig. 5.6). Greater sand eel were always observed high up in the water column in BRUVS videos with no apparent selection or use of demersal habitat, and the residual correlations plot confirms that different environmental or biotic factors were driving their correlation with other species in the study area.
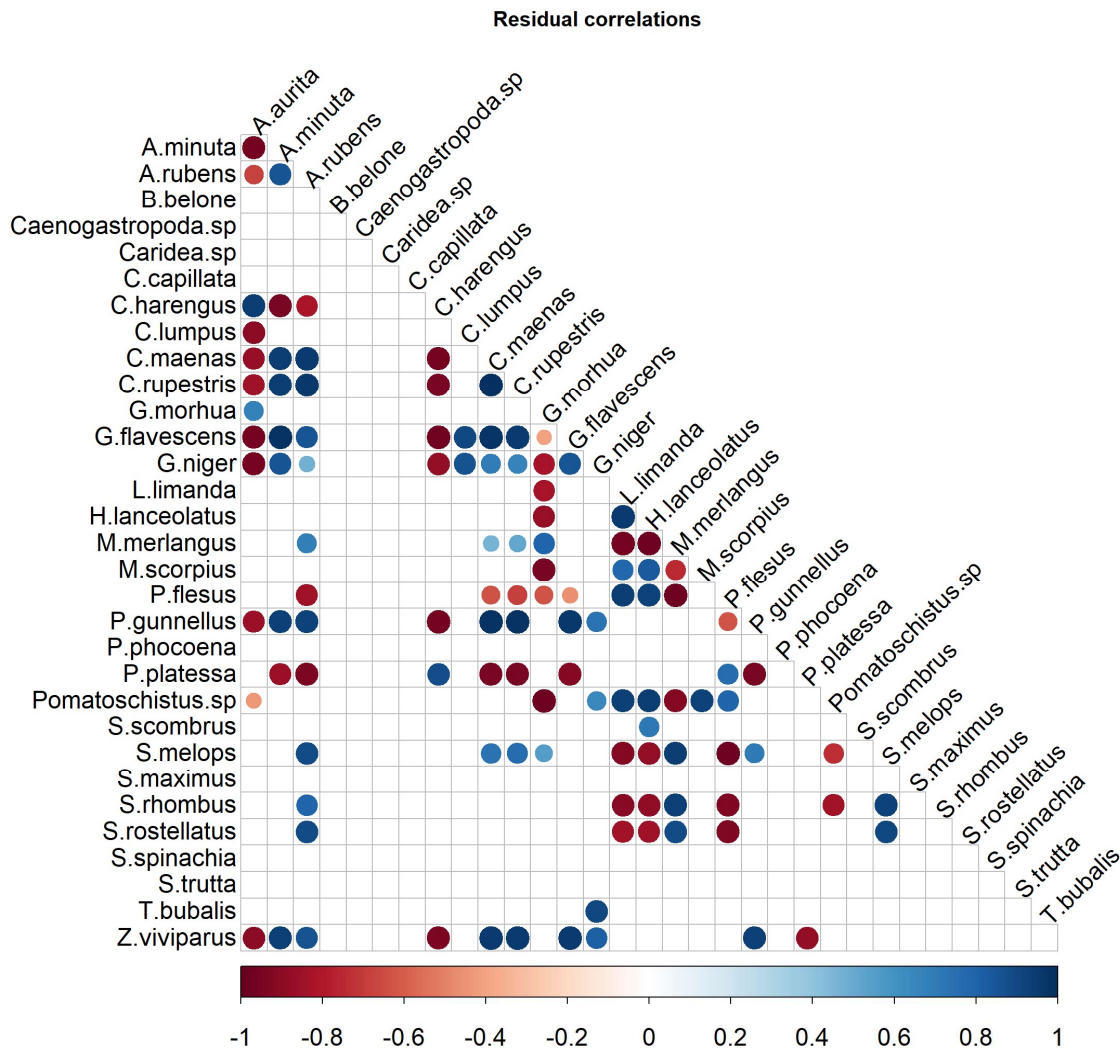
Figure 5.7 – Residual correlations from the CRM after accounting for cobble coverage on the seabed. The residual correlations can be interpreted as species correlations induced by environmental covariates not included in the CRM (in our case e.g. water temperature or salinity) or due to biotic interactions Hui (2016). See Figure 5.6 caption for further details on plot interpretation. Returning to the example described in the Figure 5.6 caption of correlations between Atlantic cod (*G. morhua*) and goldsinny wrasse (*C. rupestris*), it becomes apparent from the residual correlations plot that there are no significant residual correlations and that the strong positive association between these two species was entirely explained by cobble coverage on the seabed. Two-spotted goby (*Gobiusculus flavescens*) on the other hand, shows a significantly negative (but weak) residual correlation with cod and a strong positive residual correlation with goldsinny wrasse, which were unexplained by cobble coverage and may be caused by e.g. biotic interactions between the species or other unmeasured variables (Hui, 2016).

5.4. Discussion

We investigated the effects of cobble reef restoration on the associated marine fish and invertebrate community within a degrading coastal system of the WBS. We conducted non-invasive BRUVS monitoring, which is particularly useful for sampling within vulnerable or protected areas, like the WBS, as opposed to conventional methods (e.g. trawling) that are typically destructive to the seabed and study organisms. The univariate analysis of BRUVS recordings from the bay indicated a decrease in the posterior mean for all three Hill diversity indices and for two out of three focal taxa (with the exception of herring). Still, our BACI analysis revealed that the cobble reef restoration was to some degree effective in ameliorating these declines at the restoration sites. Most notably, we found high posterior probabilities of a positive restoration effect on Hill-Shannon and Hill-Simpson diversity relative to sand controls, while restoration effects were positive for all three Hill metrics when comparing the restored reefs to natural reefs within the bay. These results suggest that biodiversity at the restored reefs developed favorably relative to natural reefs in particular, and highlight the importance of comparing restoration sites with both a 'negative reference' (e.g. sand control) and a 'target reference' (e.g. natural reefs) to obtain a more complete picture of restoration effects (Wortley et al., 2013). The community composition at restored reefs deviated from the sand controls and appeared to resemble an intermediary composition between natural reefs and sand sites. Finally, we found no evidence of reef height driving any differences in the community of early colonizing organisms, yet the variation in coverage of cobble on the seabed induced a large amount of highly positive or negative species correlations. Combined, our results demonstrate the potential for restored cobble reefs to ameliorate declines in biodiversity and showcase the importance of understudied cobble substrates on the seabed in shaping distinct communities and driving species correlations within temperate coastal waters.

Expressing species (or taxonomic) diversity as Hill numbers of varying order (Hill, 1973; Jost, 2006) has a number of advantages relative to using traditional indices that are often used in isolation. Species richness (i.e. the number of unique species in a sample) is still by far the most commonly used species diversity metric across field studies aiming to assess trends in biodiversity within a given study area (Hellmann & Fowler, 1999; Vilmi et al., 2017; Hillebrand et al., 2018). However, species richness does not account for differences in relative abundance, dominance and rarity of species captured within the sample, and a change in biodiversity does not merely imply an increase or decrease in the number of species (Hillebrand et al., 2018). Diversity analyses are therefore often supplemented with metrics that account for species relative abundance, e.g. the Shannon or Simpson indices (Shannon, 1948; Simpson, 1949), yet their interpretation can be challenging due to non-intuitive behavior of these metrics in response to gains or losses of species. For example, as discussed in Roswell et al. (2021), an even community that losses two-thirds of its species (i.e. a 66.7% decrease in richness) may show only about half this proportional change (~ 33% decrease) in the Shannon index and even less (~10% decrease) in

the Simpson (here Gini-Simpson) index. In contrast, the Hill-Shannon and Hill-Simpson metrics (Eq. 2; Eq. 3; Roswell et al., 2021) used here all show a 66.7% proportional decline in the above case and therefore offer a more intuitive interpretation of diversity changes across time and treatments in our study. The BACI analysis using species richness indicated there was only weak evidence (~75% probability) of a positive restoration effect on richness relative to sand controls (Fig. 5.3a). Yet, expressing species diversity as Hill-Shannon and Hill-Simpson metrics revealed high probabilities of a positive restoration effect (~ 92% and 94% respectively), which combined with visual inspection of temporal trends (Fig. 5.2) indicates that the restoration of cobble habitat partly counteracted the decreases in diversity observed at sand controls. In addition, the newly restored reefs clearly developed favorably in terms of diversity (> 95% probability of positive effect for all three Hill metrics) relative to natural reefs in the area. We suggest that while species diversity declined at natural reefs (likely either due to annual variations or deteriorating conditions), the newly deployed cobble reefs offered suitable habitat for a variety of species to colonize and perhaps be released from high predation pressure by top predators (e.g. cod and harbor porpoise) associated with natural reefs (Table S5.1). However, we emphasize that our data only reflect one post-restoration period and that continued succession and development of the restored reefs is expected to strongly affect the associated reef assemblage over longer time scales, possibly to more closely resemble the composition at natural reefs. Still, these results suggest that restoration of cobble habitats likely benefits marine biodiversity and indicate that the combined use of different Hill diversity indices can yield broader insights into temporal changes in diversity across coastal systems.

The restoration of cobble reefs in this study had clear differential effects on the three focal taxa considered, i.e. cod, herring and shore crab. Cod showed a decline in posterior mean abundance across all treatment levels (control sites, natural and restored reefs) over the study period (Fig. 5.2d). Although our study only covered two time periods (i.e. two months of sampling pre- and post-restoration), implying that this trend may represent natural variations of the cod population in the bay, regional declines in condition and numbers of Baltic cod are well documented and generally attributed to a combination of climate-driven and anthropogenic factors (Eero et al., 2011; Figus, Carothers, & Beaudreau, 2017; Limburg & Casini, 2018). However, our BACI comparison provided some evidence that restored cobble reefs mitigated the observed decline in cod abundance, with an 88% posterior probability of a positive restoration effect relative to sand control sites. Cod strongly depend on hard substrate across different life stages for the provision of shelter against predators (Gotceitas & Brown, 1993), food sources (Funk, 2020) and to save energy that can subsequently be invested in somatic and gonadal growth (Schwartzbach, Behrens, & Svendsen, 2020). A recent study investigating the effects of boulder reef restoration near the current study area reported sharp increases (up to ~130-fold) in cod abundance relative to control sites (Wilms et al., 2021). In the present study, the restoration of cobble reefs did not result in a net increase in cob abundance 3-4 months post-restoration, but rather a smaller decrease in abundance (i.e. weaker slope) relative to sand controls, while the observed trend at the restored reefs resembled natural reefs (Fig. 5.2d; 5.4b). This highlights the benefit of combining the visualization of

temporal trends with computation of BACI ratios to detect and interpret effects relative to reference sites, which is imperative in systems that do not represent a 'textbook' example of a positive BACI effect (e.g. in degrading systems). We hypothesize that the low abundance of cod at the restored cobble reefs is partly due to an underdeveloped macroalgal community relative to the natural reefs in the bay. The reefs were either still completely bare or covered in thin layers of ephemeral algae, which offer substantially less structural complexity compared with perennial algae (Christie, Norderhaug, & Fredriksen, 2009). Continued colonization and growth of macroalgal species at the restored reefs is therefore expected to increase the shelter and food availability of reef-associated taxa (e.g. cod), which will need to be verified in future monitoring efforts of the sites.

Atlantic herring showed an increase in the average relative abundance over the study period across all treatment levels (Fig. 5.2e). However, the average increase appeared to be lowest at the restored reefs, which was confirmed by the low posterior probabilities (13-14%) of a positive restoration effect on herring abundance relative to sand controls and natural reefs. Herring are forage fish that are generally found high up in the water column where they feed on pelagic prey species (Wennhage & Pihl, 2002). While this may suggest that herring do not exhibit a strong preference for a particular habitat type (Rhodes et al., 2020), as also indicated by the high abundance of herring at both sand and reef sites in this study, female herring often actively select hard substrate with macroalgae to deposit their eggs during spawning (Aneer et al., 1983; Haegele & Schweigert, 1985; Šaškov et al., 2014). We did not observe herring spawning events at any of the three habitat types and thus were unable to test whether the newly restored reefs provided suitable substrate for egg deposition by herring. We argue that our BRUVS sampling method is more suitable for observing herring spawning activities than other visual monitoring methods, as movements of scuba divers (and air bubbles) associated with dive transects or diver-operated cameras are likely to disturb the spawning activity of herring. Still, grab sampling or dive surveys allow for counting and collecting of herring egg deposits (Stratoudakis, Gallego, & Morrison, 1998; Kanstinger et al., 2018), which will often be too inconspicuous to appear on BRUVS recordings unless eggs are clumped in large deposits. We hypothesize that the lack of herring spawning activity may be due to the young age of the restored reefs, since herring eggs are predominantly deposited on vegetation (Haegele & Schweigert, 1985; Kanstinger et al., 2018), which at the time of sampling was still underdeveloped. Future monitoring studies using underwater video systems within the habitat range of herring should similarly take note of unusual behavior, e.g. individuals darting vertically towards the bottom (Aneer et al., 1983), as knowledge on the occurrence of herring spawning events and associated habitat types is important to inform management strategies.

Shore crab declined in mean abundance across all treatment levels, similar to cod. Still, the BACI comparison indicated that this decline was less severe at restored reefs relative to sand controls, yielding a very high posterior probability (> 99%) of a positive restoration effect on shore crab (Fig. 5.4a). On the other hand, there was only a moderate probability (~ 59%) that the restoration effect was positive

compared with natural reefs, indicating that shore crab abundance progressed similarly over time at natural and restored reefs. Shore crabs are opportunistic omnivores that feed on a wide variety of benthic fauna including crustaceans, polychaetes and mollusks, as well as on plant material (Pihl, 1985). Notably, shore crabs can negatively impact eelgrass nursery habitats both directly through consumption of eelgrass seeds (Infantes, Crouzy, & Moksnes, 2016) or via their destructive digging behavior (Garbary, Miller, Williams, & Seymour, 2014), and indirectly through predation on mesograzers facilitating growth of ephemeral algae mats and thereby suppressing eelgrass growth (Moksnes, Gullström, Tryman, & Baden, 2008). Although eelgrass meadows were not included in the sampled areas of the current study, there is a potential for shore crab to exert similar cascading effects at cobble reefs and promote ephemeral algae over the settlement of perennial macroalgae on the reef substrate. However, shore crab is the most dominant prey item of cod in shallow areas of the study area (< 20 m; Funk et al., 2021), indicating that healthy cod populations can act as a top-down control on shore crab to prevent cascading effects from mesopredator release and strengthen the resilience of coastal habitats (Eriksson et al., 2011). We note that shore crab abundance was lowest at natural reefs, which simultaneously had the highest abundance of cod (Fig. 5.2), and that cobble coverage on the seabed induced a negative correlation between cod and crab abundance (Fig. 5.6), i.e. a large number of cod implied few shore crabs and vice versa. Combined with the high posterior probability (88%) of a positive restoration effect on cod relative to sand control sites, these results highlight the need for continued conservation and restoration of hard-bottom (e.g. cobble) habitats to prevent detrimental trophic cascades and enhance the quality of coastal ecosystems in the WBS.

Finally, we only observed one European eel (*Anguilla anguilla*) individual in the current study and were therefore unable to draw any conclusions on habitat selection or reef restoration effects for eel. The preference for hard substrate was recently shown for the elver life stage of this commercially important species in an experimental study (Christoffersen et al., 2018), which highlighted the need for conservation and active restoration of gravel and cobble habitats. However, European eels are nocturnal feeders and remain largely hidden within reefs or mud bottoms during the day and cold winter months (Nyman, 1972; van Veen, Hartwig, & Müller, 1976). Sampling in the present study took place in early spring and we only used daytime observations from our video stations, implying that any European eels present in the study area likely remained unobserved. Future studies investigating habitat use of European eel could consider using underwater video systems with artificial infrared light sources to capture their nocturnal behavior while minimizing potential effects of light-dependent motor activity (van Veen et al., 1976; Bassett & Montgomery, 2011).

We found no evidence that variations in reef height had any effect on the fish and invertebrate community associated with the restored reefs. While the importance of variability in vertical relief within reef systems has been documented extensively (Rilov & Benayahu, 2000; Granneman & Steele, 2015; Paxton, Pickering, Adler, Taylor, & Peterson, 2017; Komyakova, Chamberlain, Jones, & Swearer, 2019),

the effect of differences in relative height between reefs of similar topography has received less attention. Wilhelmsson et al. (2006) manipulated the height of PVC pipes used as vertical structures in their artificial reefs, with heights of 1 m and 3 m comparable to the 0.6 m and 1.3 m used in the present study, and similarly found no effect on the associated fish community. Potential implications of constructing cobble reefs to protrude high up in the water column include higher detection rates by roaming pelagic species (Wilhelmsson et al., 2006) and elevated light intensities for the growth of algal communities. On the other hand, the construction of low reefs required only half of the cobble that was required to construct the high reefs in the present study. However, at the time of sampling, the cobble reefs had only been restored for 3-4 months and it would be safe to assume that, if height plays an important role in shaping the community structure on reefs, differential algal growth on high and low reefs would need ample time (i.e. years) to develop and require long-term monitoring to be evaluated. We are therefore currently unable to provide recommendations on optimal reef height based on our initial post-restoration monitoring efforts, and note that future studies based on ongoing monitoring of the restoration sites are likely to provide further insights on the effects of reef height on colonizing marine species.

Collectively, this study provides evidence that fish and invertebrate communities differ between cobble reefs and bottoms covered by sand. This conclusion is only valid, however, for the larger fractions of cobble (rock diameter > 20 cm) as the present study did not investigate effects of smaller fractions of cobble (rock diameter < 15 cm). Our findings suggest that removal of large cobble from the seabed may impact local species diversity, community composition and the co-occurrence of coastal species. Moreover, our study shows that restoration of cobble reefs is feasible, although the outcome is not directly comparable with natural cobble reefs within the time frame tested by the present study (½ year post-restoration). Specifically, Atlantic cod and herring were on average more abundant on established natural cobble habitat compared to our newly deployed reefs, although the observed temporal change in cod abundance was similar between natural and restored reefs, and differed from the sand sites. We examined the hypothesis that adult herring would utilize restored cobble reefs for spawning. Increased abundance of spawning herring, and the developing eggs (embryos) in particular, could attract larger predators preying on the adult herring and their eggs. The larger predators could include cod and other reef-associated taxa; however, we found no evidence of spawning herring in the restored reefs. During the field study, spawning herring were captured by local fishermen in the area, but the fish were apparently not utilizing the restored reefs as spawning locations. BRUVS recorded a relatively high abundance of herring at natural reefs (although slightly lower than at sand sites), suggesting that herring are to some degree associated with natural reef areas and might be spawning at night or perhaps in shallower waters than examined by the present study. We recommend that the restored cobble reefs are examined again when the reefs are approaching full colonization by sessile and slowly growing organisms, including different species of macroalgae. Finally, further studies are required to understand the importance of the small fractions of cobble, pebble and gravel, which are currently being removed from the seabed in dedicated areas in Denmark.

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Supporting information

Table S1 – Complete list of species observations from the video recordings. Observations are grouped per time level (i.e. before and after the restoration) and treatment level (i.e. control, natural and restored reefs). The values for each observation indicate the frequency of occurrence, i.e. the percentage (0-100%) of samples (continuous BRUVS recordings) for which the given species was observed. The sampling effort (N) is provided in the column headers for each treatment level before and after the reef restoration. Values in brackets correspond to the mean MaxN (i.e. maximum number of individuals within a video frame) for those samples in which the given species was observed.

Species observed			Pre-restoration			Post-restoration		
Family / Order	Scientific name	Common name	Sand Control (N=44)	Natural Reef (N=47)	Restored Reef (N=43)	Sand Control (N=66)	Natural Reef (N=67)	Restored Reef (N=112)
Anguilliformes	*Anguilla anguilla*	European eel	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.89 (1)
Agonidae	*Agonus cataphractus*	Hooknose	0 (0)	0 (0)	0 (0)	1.52 (1)	0 (0)	0 (0)
Ammodytidae	*Hyperoplus lanceolatus*	Greater sandeel	0 (0)	0 (0)	0 (0)	0 (0)	1.49 (5)	0.89 (1)
	Ammodytidae sp.	Unidentified sandeel	0 (0)	0 (0)	0 (0)	12.1 (15.6)	8.96 (28.5)	33.0 (55.8)
Annelida	*Annelida* sp.	Unidentified annelid	0 (0)	0 (0)	0 (0)	1.52 (1)	0 (0)	0 (0)
Asteriidae	*Asterias rubens*	Common starfish	93.2 (3.49)	76.6 (2.39)	95.3 (3.20)	50 (3.21)	38.8 (1.35)	49.1 (2.98)
Belonidae	*Belone belone*	Garfish	0 (0)	0 (0)	0 (0)	1.52 (5)	0 (0)	0.89 (1)
Buccinidae	*Buccinum undatum*	Common whelk	4.55 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Caenogastropoda	*Caenogastropoda* sp.	Unidentified snail	11.4 (4.2)	10.6 (1)	4.65 (1)	7.58 (2.6)	0 (0)	0 (0)
Caridea	*Crangon crangon*	Common shrimp	0 (0)	2.13	0 (0)	4.55 (1)	0 (0)	0 (0)
	Caridea sp.	Unidentified shrimp	0 (0)	2.13 (1)	0 (0)	1.52 (1)	0 (0)	8.04 (1.11)
Clupeidae	*Clupea harengus*	Atlantic herring	65.9 (65.1)	57.4 (24)	62.8 (37.5)	69.7 (53.4)	53.7 (34.8)	30.4 (52.6)
	Clupeidae sp.	Unidentified clupid	0 (0)	0 (0)	0 (0)	12.1 (77.1)	23.9 (54)	13.4 (58.8)
Cottidae	*Myoxocephalus scorpius*	Shorthorn sculpin	4.55 (1)	2.13 (1)	0 (0)	4.55 (1)	1.49 (1)	8.93 (1)
	Taurulus bubalis	Longspined bullhead	0 (0)	0 (0)	0 (0)	1.52 (1)	0 (0)	5.36 (1)
	Cottidae sp.	Unidentified sculpin	0 (0)	0 (0)	0 (0)	0 (0)	1.49 (1)	10.7 (1)
Cyaneidae	*Cyanea capillata*	Lion's mane jellyfish	0 (0)	0 (0)	0 (0)	3.03 (1)	0 (0)	0.89 (1)
Cyclopteridae	*Cyclopterus lumpus*	Lumpfish	0 (0)	2.13 (1)	0 (0)	1.52 (1)	0 (0)	0.89 (1)
Gadidae	*Gadus morhua*	Atlantic cod	100 (1.52)	100 (6.19)	72.1 (1.13)	51.5 (3.9)	100 (14.5)	53.6 (5.5)
	Pollachius virens	Saithe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.89 (1)
	Merlangius merlangus	Whiting	100 (8.09)	51.1 (5.67)	81.4 (4.4)	9.09 (57)	1.50 (1)	1.79 (1)
	Pollachius sp.	Unidentified pollack	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.89 (1)
	Gadidae sp.	Unidentified gadoid	13.6 (1.17)	17.0 (3.88)	11.6 (1)	1.52 (1)	19.4 (1.92)	8.03 (1.11)
Gasterosteidae	*Spinachia spinachia*	Fifteen-spined stickleback	2.27 (1)	0 (0)	2.33 (1)	6.06 (1)	5.97 (1)	2.68 (1)
Gobiidae	*Aphia minuta*	Transparent goby	0 (0)	0 (0)	0 (0)	1.52 (1)	2.99 (1.5)	4.46 (2.8)
	Gobius niger	Black goby	0 (0)	2.13 (1)	0 (0)	4.55 (1)	1.49 (1)	12.5 (1.07)
	Gobiusculus flavescens	Two-spotted goby	4.55 (1)	72.3 (2.68)	4.65 (1)	24.2 (8.1)	61.2 (1.9)	52.7 (9.6)
	Pomatoschistus sp.	Sand goby	13.6 (1.17)	4.26 (1.5)	16.3 (1.14)	72.7 (1.73)	7.46 (1.2)	42.9 (1.73)
	Gobiidae sp.	Unidentified goby	31.8 (1.07)	85.1 (1.5)	44.2 (1.11)	4.55 (1)	10.4 (2.29)	4.46 (2.6)
Labridae	*Ctenolabrus rupestris*	Goldsinny wrasse	13.6 (1.33)	100 (1.76)	4.65 (1.5)	10.6 (1.29)	50.7 (2.18)	56.3 (2.32)
	Symphodus melops	Corkwing wrasse	0 (0)	17.0 (1)	0 (0)	1.52 (1)	0 (0)	0 (0)
	Labridae sp.	Unidentified wrasse	0 (0)	23.4 (1.36)	2.33 (1)	0 (0)	7.46 (1.4)	5.36 (1.83)
Littorinidae	*Littorina littorea*	Common periwinkle	6.82 (2)	0 (0)	2.33 (1)	0 (0)	0 (0)	0 (0)
Mugilidae	*Chelon labrosus*	Thicklip grey mullet	2.27 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nassariidae	*Tritia reticulata*	Netted dog whelk	0 (0)	0 (0)	0 (0)	6.06 (1.25)	0 (0)	0 (0)
	Nassariidae sp.	Unidentified whelk	0 (0)	0 (0)	0 (0)	1.52 (1)	0 (0)	0 (0)
Phalacrocoracidae	*Phalacrocorax carbo*	Great cormorant	2.27 (1)	2.13 (1)	0 (0)	0 (0)	1.49 (1)	0.89 (1)

Phocidae	*Phoca vitulina*	Harbour seal	0 (0)	0 (0)	0 (0)	1.52 (1)	0 (0)	0 (0)
Phocoenidae	*Phocoena phocoena*	Harbour porpoise	4.55 (1)	2.13 (1)	0 (0)	7.58 (1.4)	14.9 (1.2)	0.89 (1)
Pholidae	*Pholis gunnellus*	Rock gunnel	11.4 (1)	23.4 (1)	0 (0)	10.6 (1.29)	19.4 (1.15)	5.36 (1)
Pleuronectidae	*Limanda limanda*	Common dab	6.82 (1.6)	0 (0)	11.6 (1.6)	25.8 (1.06)	4.48 (1)	28.6 (1.22)
	Platichthys flesus	European flounder	11.4 (1.2)	0 (0)	6.98 (1.33)	56.1 (1.49)	8.96 (1.17)	53.8 (1.08)
	Pleuronectes platessa	European plaice	13.6 (1.33)	0 (0)	25.6 (1.27)	37.9 (1)	2.99 (1)	18.8 (1.05)
	Pleuronectidae sp.	Unidentified right-eyed flatfish	0 (0)	0 (0)	0 (0)	100 (2.26)	13.4 (1.11)	49.1 (1.44)
Pleuronectiformes	*Pleuronectiformes* sp.	Unidentified flatfish	100 (1.44)	21.3 (1)	100 (1.67)	37.9 (1.04)	7.46 (1)	50 (1.05)
Portunidae	*Carcinus maenas*	Shore crab	100 (10)	89.4 (2.76)	97.7 (6.55)	69.7 (4.54)	58.2 (2.41)	84.8 (5.21)
Salmonidae	*Salmo trutta*	Brown trout	2.27 (1)	2.13 (1)	2.33 (1)	1.52 (1)	10.4 (1.14)	6.25 (1)
	Salmonidae sp.	Unidentified salmon	0 (0)	0 (0)	0 (0)	0 (0)	1.49 (1)	0 (0)
Scombridae	*Scomber scombrus*	Atlantic mackerel	0 (0)	0 (0)	0 (0)	1.52 (25)	1.49 (4)	0.89 (4)
Scophthalmidae	*Scophthalmus maximus*	Turbot	0 (0)	0 (0)	0 (0)	3.03 (1)	1.49 (1)	4.46 (1)
	Scophthalmus rhombus	Brill	6.82	0 (0)	0 (0)	1.52		0 (0)
	Scophthalmidae sp.	Unidentified left-eyed flatfish	0 (0)	0 (0)	0 (0)	3.03 (1)	0 (0)	0.89 (1)
Syngnathidae	*Nerophis ophidion*	Straightnose pipefish	0 (0)	0 (0)	0 (0)	1.52 (1)	0 (0)	0 (0)
	Syngnathus rostellatus	Nilsson's pipefish	0 (0)	4.26 (1)	2.33 (1)	1.52 (1)	0 (0)	0 (0)
	Syngnathus acus	Greater pipefish	0 (0)	0 (0)	0 (0)	1.52 (1)	0 (0)	0 (0)
Ulmaridae	*Aurelia aurita*	Moon jelly	2.27 (1)	4.26 (1)	0 (0)	6.06 (1)	11.94 (1.25)	25.89 (1.28)
Zoarcidae	*Zoarces viviparus*	Common eelpout	4.55 (1)	17.0 (1)	0 (0)	6.06 (1.25)	28.4 (1.47)	19.6 (1)

Chapter 6

Environmental DNA reveals fine-scale habitat associations for resident and sedentary marine species across a mosaic of soft and hard-bottom habitats*

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Abstract

The pressure on coastal marine habitats is intensifying at an accelerating pace and the capacity for management strategies to adapt relies strongly on accurate scientific knowledge about spatiotemporal distributions of marine species and their association with coastal habitats. Traditional survey techniques are typically costly and may inflict additional negative impacts to the study system through habitat destruction or invasive sampling of study organisms. Here, we investigate the potential of an emerging low-cost and non-invasive technique, environmental DNA (eDNA) sampling, to detect species-habitat associations (SHAs) for four common coastal species in the western Baltic Sea. We performed a direct comparison between species-specific qPCR analysis and baited remote underwater video system (BRUVS) samples, a more established yet similarly non-invasive method widely used to monitor marine habitats. Focal species (cod *Gadus morhua*, flounder *Platichthys flesus*, plaice *Pleuronectes platessa* and goldsinny wrasse *Ctenolabrus rupestris*) were selected based on differential life styles (i.e. resident vs transient) and mobility levels (i.e. sedentary vs highly mobile), to investigate whether these factors might affect the detection of SHAs from eDNA on a local scale. Accordingly, a species-specific qPCR assay is developed and tested for the detection of eDNA from goldsinny wrasse, which is made available herein. In addition, we examined potential correlations between eDNA signals and abundance counts (MaxN) from subsequently deployed BRUVS, to assess the potential for estimating fish abundance from eDNA samples. Results obtained from Bayesian multi-level models indicated that eDNA sampling provided strong evidence for a sand association for flounder (98% posterior probability) and a reef association for wrasse (99% posterior probability), respectively, in agreement with results obtained from BRUVS. However, while BRUVS concurrently provided strong evidence for SHAs for cod and plaice, eDNA sampling did not detect any habitat association for these species. Since flounder and plaice exhibit similar sedentary lifestyles, we suspect that a lack of any association for plaice was due to generally low plaice eDNA concentrations in the study area that may have precluded statistical inference on habitat effect. Finally, although we found evidence for a positive correlation between wrasse eDNA detection and abundance on videos (posterior probability 95%), evidence for similar correlations was lacking for the remaining species and the explanatory power of all relationships was generally low. We hypothesize that the positive correlation for wrasse originates from its highly conspicuous appearance (making wrasse unlikely to be missed or misidentified in videos) combined with its high residency rate within small-scale habitat patches that may imply highly localized shedding of eDNA material relative to more transient species. Collectively, our results demonstrate that eDNA sampling is capable of detecting SHAs on a fine spatial scale, yet this ability likely depends on the mobility and lifestyle of study organisms, with SHAs for species exhibiting small home ranges or a sedentary lifestyle most likely to be accurately described. Combined use with traditional survey methods is advised to improve the detection of SHAs for highly mobile and transient species, or for species with very low eDNA concentrations. We also recommend that future studies expecting low species concentrations should sample water volumes > 1 L to enhance detection rates of target species and potentially facilitate a quantitative assessment of eDNA data.

6.1. Introduction

Coastal marine habitats offer numerous ecosystem services that can benefit societal wellbeing and support critical ecological links with surrounding environments (Barbier et al., 2011; Lau, Hicks, Gurney, & Cinner, 2019; Duarte et al., 2020). For example, coastal reefs act as important nursery grounds for juveniles fishes and invertebrates, while offering benefits in the form of coastal protection, fisheries and recreational values (Barbier, 2017; Lefcheck et al., 2019). Safeguarding coastal habitats from cumulative human impacts requires adaptive management strategies that are guided by accurate scientific information. This includes knowledge on spatiotemporal distributions of marine organisms and their association with the surrounding habitat, which provides insights into species-specific responses to environmental change and allows for effective conservation efforts (Espinoza, Araya-Arce, Chaves-Zamora, Chinchilla, & Cambra, 2020). Traditional catch surveys can be destructive to the environment, costly to perform and highly selective in sampled taxa and size classes (Murphy & Jenkins, 2010). While all marine monitoring techniques are recognized to have their own intrinsic biases and limitations (Murphy & Jenkins, 2010; Danovaro et al., 2016), there is a growing demand for standardized cost-effective methods that enable low-impact assessments of species distributions across a diverse range of marine systems.

A common sampling method is the use of baited remote underwater video stations (BRUVS), offering non-invasive assessment of aquatic environments from video recordings that can be permanently archived and shared within global repositories (Langlois et al., 2020). BRUVS are used to document a wide variety of marine organisms from the tropics to the polar regions, with well-established protocols on system design, sampling methodology and data processing (Whitmarsh, Fairweather, & Huveneers, 2017; Langlois et al., 2020). The non-destructive nature of BRUVS sampling, with minimal disturbance to marine life and the seabed, is particularly useful for monitoring vulnerable species and habitats (e.g. endangered species in protected areas; Espinoza et al., 2020). BRUVS sampling allows for the recording of marine community metrics, including relative species abundance, diversity indices, community composition and behavioral aspects (e.g. predator-prey interactions and spawning events). In addition, the stereo-video adaptation of BRUVS enables observers to derive fish length-frequency distributions and estimate the biomass of the sampled fish community using established length-weight relationships (Langlois et al., 2020). The use of bait to attract individuals to the camera's field of view reduces between-sample variability in species counts and the risk of false negatives, thereby promoting the power of statistical inference from BRUVS data, relative to unbaited cameras (Bernard & Götz, 2012). Several limitations associated with use of bait attractants remain important to consider, including a possible underrepresentation of herbivores in BRUVS samples and potential difficulties in discriminating between fine-scaled community patterns when attracting species from surrounding habitats. While several studies employing BRUVS have indeed reported higher piscivore abundances relative to unbaited methods (Harvey, Cappo, Butler, Hall, & Kendrick, 2007; Goetze et al., 2015), herbivores generally remain well

represented in BRUVS samples (Harvey et al., 2007; Watson, Harvey, Fitzpatrick, Langlois, & Shedrawi, 2010). In addition, the use of bait often results in better discrimination of fish assemblages (Harvey et al., 2007) and more robust species-habitat associations (SHAs) relative to unbaited cameras (Rhodes et al., 2020). BRUVS sampling has therefore become an established marine monitoring technique, yet sampling and analysis of the video data remain costly and time consuming and the data quality highly depends on the taxonomic expertise of video observers.

Monitoring marine communities from environmental DNA (eDNA) has gained significant interest since the pioneering study by Thomsen et al. (2012), which showed that it is possible to detect macro-organisms from eDNA molecules in seawater. Marine organisms continuously release DNA particles into the surrounding water through scales, skin tissue, feces, metabolic waste, gametes and carcasses (Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012; Thomsen & Willerslev, 2015; Takeuchi et al., 2019). From the DNA material, species-specific detection can be achieved using real time quantitative polymerase chain reaction (qPCR), which involves measuring amplification-dependent increases in fluorescence signals from targeted species assays (Heid, Stevens, Livak, & Williams, 1996; Brian Klitgaard Hansen, Bekkevold, Clausen, & Nielsen, 2018) . Alternatively, a DNA metabarcoding approach enables broad assessments of community structure and biodiversity in marine systems, providing a taxonomic breadth unparalleled by any other single sampling method (Stat et al., 2017). Given the opportunity to rapidly obtain large sample sizes with a high sensitivity for species detection, there is strong interest from a conservation and fisheries management perspective in exploring the potential for inferring fish abundance, and eventually population status, from eDNA data. Previous studies have confirmed that the amount of released DNA material correlates to both the size (Maruyama, Nakamura, Yamanaka, Kondoh, & Minamoto, 2014) and number of individuals (Lacoursière-Roussel, Rosabal, & Bernatchez, 2016) in controlled aquatic environments. While there is some evidence that the correlation holds for natural systems (Lacoursière-Roussel, Côté, Leclerc, & Bernatchez, 2016; Doi et al., 2017), explanatory power of the relationship becomes substantially lower due to inherent fluctuations in environmental conditions (Yates, Fraser, & Derry, 2019). For example, variations in water temperature or pH can affect the production and degradation of eDNA molecules (Strickler, Fremier, & Goldberg, 2015; Hansen et al., 2018) and thereby alter the relationship between eDNA concentration and species abundance. Direct comparisons with abundance metrics from traditional methods can shed further light on such species-specific relationships, in particular if abundance is concurrently recorded at the same sampling location. BRUVS sampling offers an opportunity in this regard, as BRUVS can be rapidly deployed at the same location, while the bait may attract individuals in the vicinity that were captured in the eDNA 'snapshot'. Inter-method comparisons between eDNA and BRUVS have thus far focused on biodiversity and community assessments (Boussarie et al., 2018; Stat et al., 2019; Jeunen et al., 2020; Mercaldo-Allen et al., 2021), yet potential correlations between species-specific qPCR detection and abundances recorded from BRUVS remain, to the best of our knowledge, untested.

Currently, inferences based on BRUVS or eDNA surveys are limited by their undefined spatial resolution. In BRUVS, the soaking bait produces a bait plume that may attract individuals from considerable distances to the stationary camera system, effectively rendering the sampled area to be unknown. Efforts have been made to estimate bait plume size based on current velocity (Heagney, Lynch, Babcock, & Suthers, 2007; Taylor, Baker, & Suthers, 2013), yet accurate description of the sampled area would ultimately require complex three-dimensional hydrodynamic modelling and depend on odor detection thresholds of target species (Westerberg & Westerberg, 2011; Taylor et al., 2013). Similarly, eDNA molecules are transported by ocean currents, leading to uncertainty about the origin of the DNA material and therefore the spatial explicitness of eDNA data (Roussel, Paillisson, Tréguier, & Petit, 2015). Still, there is growing evidence that eDNA surveys can differentiate between fine-scaled patterns in community assemblages (Port et al., 2016; Stat et al., 2019; West et al., 2020), even across a wide geographical extent (700 km of coastline; West et al., 2021). While such localized signals may indicate high site fidelity of eDNA to the source community (Harrison, Sunday, & Rogers, 2019), variations in mobility across marine species could affect the ability of eDNA sampling to detect SHAs, which has thus far received little attention.

Here, we investigated the potential of BRUVS and eDNA to effectively record habitat associations for four temperate marine species on a small spatial scale ($\sim$ 20 km^2) in the western Baltic Sea (WBS). We selected two sand-associated and two reef-associated fish species, ranging from sedentary to highly mobile and from resident to transient, and assessed whether each sampling method could detect the respective habitat associations. In addition, we investigated potential correlations between relative fish abundance recorded by BRUVS and eDNA signals in the seawater samples. Specifically, we tested three hypotheses. First, we expected BRUVS and eDNA to detect comparable habitat associations for the four focal species, i.e. higher abundance of reef fishes recorded on reefs relative to sand and vice versa for sand species (H1). Second, species detection rates (i.e. presence-absence signals) were expected to be higher for cryptic benthic species when using eDNA, but similar between methods for a highly conspicuous and mobile predatory fish (H2). Lastly, we hypothesized that eDNA signals are positively correlated with relative abundance of the focal species as recorded by BRUVS (H3).

6.2. Materials and Methods

6.2.1. Study area

The study was conducted in Sønderborg Bay within Flensborg Fjord, a Natura 2000 designated protected area shared between Denmark and Germany. The seabed of the bay consists mainly of muddy sand, with patches of fine sand along the coastline occasionally covered by glacial till ranging from gravel to boulders (Fig. 6.1). We sampled four different field sites with depths between 6-7 m: Kegnæs Ende (SE)

and Viemose (NW) represented soft bottom habitats, while Spar Es (SW) and Vesterhage (N) were characterized by hard-bottom glacial till. Sampling focused on four relatively abundant marine fish species with documented habitat associations. Atlantic cod (*Gadus morhua*; hereafter "cod") and goldsinny wrasse (*Ctenolabrus rupestris*; hereafter "wrasse") are reef-associated fish species from the area, while European flounder (*Platichthys flesus*; hereafter "flounder") and European plaice (*Pleuronectes platessa*; hereafter "plaice") are associated with soft sediment habitats (Rhodes et al., 2020).
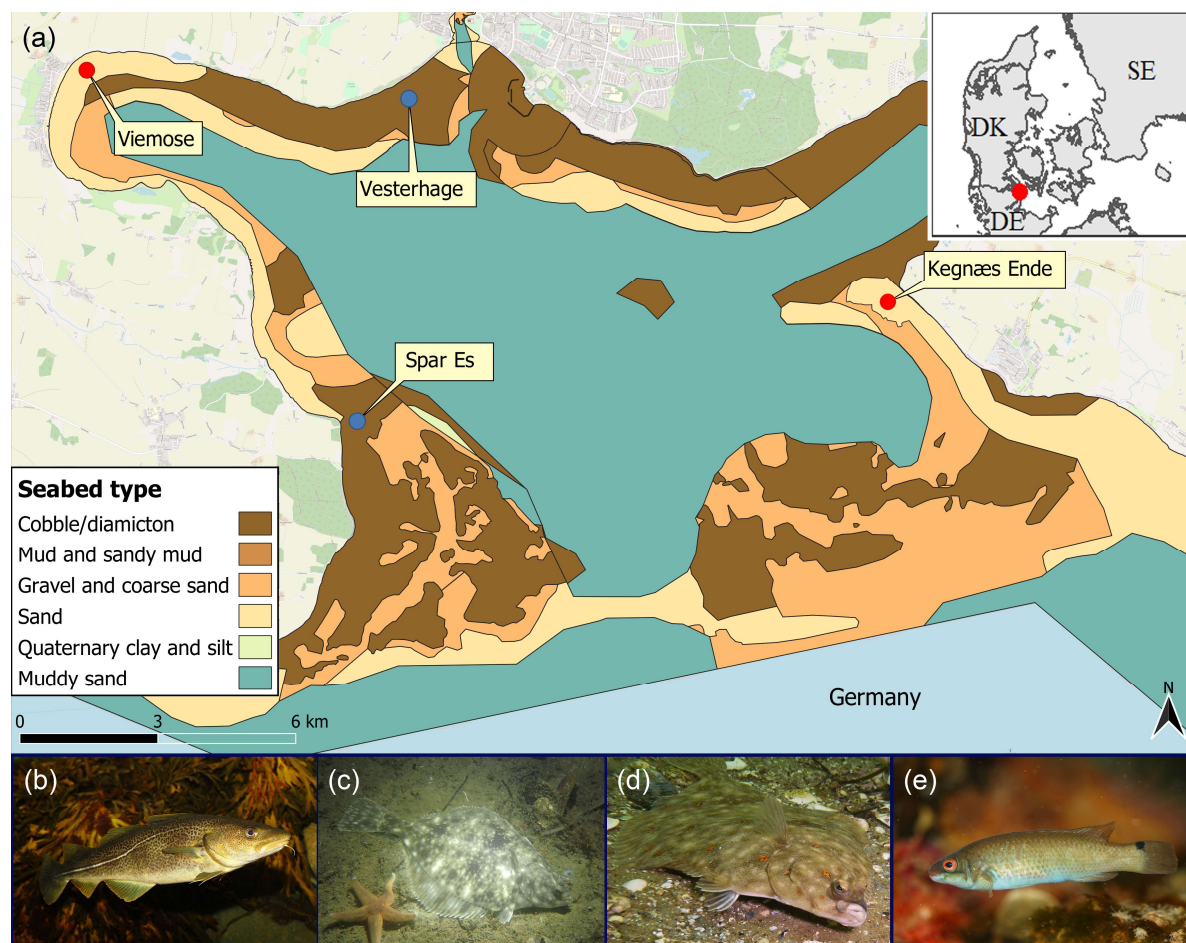
Figure 6.1 – Map of the study location in the western Baltic Sea (WBS). The seabed structure and composition has gradually been shaped by glacial erosion and deposition, resulting in a mosaic of different seabed types across the bay (a). Glacial till deposits constitute the main type of hard substrate within the bay, also classified as diamicton; an unsorted mixture of sediment with particle sizes ranging from small pebbles to boulders. However, the bay is generally dominated by soft sediment, including extensive areas of fine sand and muddy sand bottom. Paired BRUVS and eDNA samples were collected from sand sites (blue circles) and reef sites (red circles), to document habitat associations for (b) Atlantic cod (*Gadus morhua*), (c) European flounder (*Platichthys flesus*), (d) European plaice (*Pleuronectes platessa*) and goldinnsy wrasse (*Ctenolabrus rupestris*). Photo credits: Nicolai Ulrik Hansen (b; e), Micha Baum (c) and Rita Jansen (d). Background data of sediment map obtained from GEUS (Leth, 2021).

6.2.2. Field sampling

Sampling took place from late April until late June of 2018, during which sea surface temperatures (SST) increased from 6.4˚C to 20.8˚C in the study area. Water samples were collected directly from the surface water and extracted using a 60 mL sterile syringe and injected into an enclosed Sterivex-GP capsule filter (0.22 μm pore size, SVGPL10RC, Millipore, CA, USA). A total of 1000 mL seawater was filtered for each sample, however on one occasion, due to clogging, only 850 mL could be filtered. After filtration, the sterivex filters were stored in a cooling box on ice and subsequently transferred to a -20˚C freezer upon return from the field. A maximum of two samples were taken at different sites on a given sampling day.

Immediately following the eDNA water sampling, we deployed two BRUVS at the same field site. BRUVS consisted of GoPro's Hero 3 or 4 (GoPro, USA), attached to a steel pole (1 m height; 3 cm diameter) at 20 cm above the seabed and positioned firmly on a concrete tile with the camera field of view parallel to the seabed. BRUVS deployments were separated by at least 100 m to minimize the risk of double counting individuals between the two BRUVS while staying within the designated habitat type. We used chopped Atlantic herring (*Clupea harengus*) as bait, packed tightly in a mesh bait bag. The bait bag was positioned 15 cm above the seabed and attached via an 80 cm bait arm in the lower center of the field of view. We ensured at least 48 h between consecutive sampling at a particular site, to avoid contamination from previously deployed BRUVS and ensure that sites had time to normalize. Soak time of the BRUVS varied between 1h – 2h 40min depending on the camera's battery life, with a small number of BRUVS (< 5) marked unsuccessful due to battery failure or loss of the bait bag. Accordingly, a total of 41 seawater samples were taken for eDNA extraction and coupled with 78 BRUVS deployments across the four different field sites (Fig. 6.1).

6.2.3. Video analysis

We analyzed the video recordings using VLC media player (https://www.videolan.org/vlc/). The four focal species were identified and counted by trained video observers with use of morphological characteristics and reference images. Individuals that were challenging to identify to species level (e.g. due to distance from the camera or poor visibility) were instead labelled on a genus or family level and omitted from the present study. We expressed the abundances of focal species as the maximum number of individuals per species in a single video frame (MaxN), a metric widely used to avoid duplicate counts (Cappo, Speare, & De'Ath, 2004). In addition, video observers estimated the functional visibility in each video clip from taped markers on the bait pole and by comparing the field of view to reference images.

6.2.4. Quantitative PCR

All DNA extractions and setup of quantitative PCR (qPCR) reactions were conducted in a dedicated clean laboratory facility at DTU Aqua (Technical University of Denmark, Silkeborg, Denmark). DNA extraction was based on a modified version of a previously published eDNA extraction protocol (Spens et al., 2017). This protocol uses the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) to extract eDNA directly from the Sterivex filters (Supporting information, Note S1). Each extraction batch included a negative control (extraction blank) to test for exogenous DNA contamination through the used reagents or from the laboratory. Final DNA concentration was measured for every sample using a Qubit™ flourometer and the Qubit™ dsDNA high sensitivity kit (ThermoFisher Scientific, Waltham, MA, USA).

Four different qPCR assays targeting the mitochondrial cytochrome b gene (*cytb*) were used to analyze eDNA from the collected filters (Table 6.1). Assays for cod, flounder and plaice were based on published species-specific primers and probes (Knudsen et al., 2019) while the assay for goldsinny wrasse was designed for the study. This assay was developed based on thorough *in silico* and *in vitro* tests to ensure specificity. In order to ensure a broader use of the assay, the *in silico* analysis included all closely related species of wrasse with an available genetic *cytb* sequence in Genbank (https://www.ncbi.nlm.nih.gov/genbank/). After database establishment, all primers and probes were aligned to the reference sequences using Geneious Prime® 2019.0.4 (https://www.geneious.com/) to verify specificity (Supporting Information, Table S6.1). Specificity was further tested *in vitro* via qPCR on 1 ng of extracted DNA from the target species, as well as Ballan wrasse (*Labrus bergylta*) and Corkwing wrasse (*Symphodus melops*), which are the only other wrasse species potential occurring in the sampling area (personal communication, Peter Rask Møller, Natural history Museum of Denmark). Finally, the assay targeting goldsinny wrasse was optimized for sensitivity by testing combinations of primers (200, 400, 600 and 800 nM) and probes (200, 300 and 400 nM) on 1 ng of target DNA. The combination showing the lowest Ct-value (cycle-threshold) was chosen for qPCR analysis.

Quantitative PCR reactions were performed in 20 µL reaction volumes containing the optimal concentration of primers and probe (Table 6.1) with 8 µL of TaqMan Environmental Master Mix 2.0 (ThermoFisher Scientific, Waltham, MA, USA) and 4 µL DNA template. All qPCR reactions were analyzed on a StepOne Plus Real-time PCR instrument (Life Technologies, Carlsbad, CA, USA) using the following thermal cycling profile: 5 min at 50 °C and 10 min initial denaturation at 95°C, followed by 50 cycles of 95°C for 15 seconds and 60°C for 1 min. Initially, all samples were analyzed using a positive control (IPC, Applied Biosystems) to ensure that potential PCR inhibitors did not interfere with the PCR reaction. Subsequently, all samples were run in triplicates. These included one field blank, two extraction blanks, and six NTCs per analyzed species. The number of DNA copies per reaction was estimated using a dilution series from $1-1\times10^5$ copies of DNA targets. DNA Amplicons were generated by conventional PCR using the qPCR primers and were purified using the Nucleospin Gel and PCR clean-up kit (Macherey-

nagel, Düren, Germany). Their concentrations were measured using a Qubit™ flourometer and the Qubit™ dsDNA High sensitivity kit to enable dilution to precise copy number.

All qPCR results were compared to a standard dilution series and categorized in terms of limit of detection (LOD) and limit of quantification (LOQ). We defined LOD as the lowest copy number detected in at least one of the triplicates in the standard dilution series and LOQ as the lowest copy number detected in all triplicates in the standard dilution series (Ellison, English, Burns, & Keer, 2006). However, most qPCR estimates of copy numbers were below LOQ due to low DNA concentration of the four targeted species in the collected samples (see Results). Thus, as a proxy for DNA concentration we used the proportions of positive amplifications of the individual samples, as it is reasonable to expect that amplification rates increase with the relative concentration of target DNA molecules (Furlan et al., 2016).

Table 6.1 – Assay information for the four assays used in this study.

* To ensure high sensitivity all assays have been optimized for optimal concentration of primer and probes.

Assay	Sequence	Length (bp)	Concentration (Nm)*	Reference
Atlantic cod (*Gadus morhua*) – 80 bp of mtCytB				
Gadmor_F15076	TTCGCACCTAATTTACTCGGAG	22	400	Knudsen et al. (2019)
Gadmor_R15155	TCGGGCTTAACATGAGGTGG	20	800	Knudsen et al. (2019)
Gadmor_P15102	FAM-AGATAATTTCACCCCTGCTAACCCCATC-BHQ1	28	300	Knudsen et al. (2019)
European flounder (*Platichthys flesus*) – 88 bp of mtCytB				
Plafle_F15107	TAGGCTTTGCAGTTCTCCTT	20	200	Knudsen et al. (2019)
Plafle_R15194	GCAGGCGTAAAGTTGTCCG	19	800	Knudsen et al. (2019)
Plafle_P15131	FAM-CACTGGCTTCGCTCGCCCTATTTTC-BHQ1	26	300	Knudsen et al. (2019)
European plaice (*Pleuronectes platessa*) – 90 bp of mtCytB				
Plepla_F15107	TAGGCTTCGCAGTCCTCCTC	20	1000	Knudsen et al. (2019)
Plepla_R15196	TTGCAGGCGTGAAGTTGTCT	20	200	Knudsen et al. (2019)

Plepla_P15169	FAM-CTAAAAGATTTGGGGAAAATAGGGCGAGT-BHQ	29	300	Knudsen et al. (2019)
Goldsinny wrasse (*Ctenolabrus rupestris*) – 138 bp of mtCytB				
CterupR_F705	TCTCATCGCCCTAACTTCCCTA	22	600	This study
Cterup_R842	AGGATGGCATAAGCAAACAGGA	22	800	This study
Cterup_P791	FAM-CCCCACCG/ZEN/CACATTAAACCAGAGTGG	26	400	This study

6.2.5. Statistical analysis

All analyses were performed in R statistical software version 3.6.3 (R Core Team, 2020). We used a Bayesian multilevel modelling approach to obtain a probabilistic interpretation to our study hypotheses (Gelman et al., 2013). All models were fitted using the *brms* package (Bürkner, 2017), which facilitates Bayesian model fitting through the R interface of the STAN programming language (Carpenter et al., 2017). We used four Markov Chains for each model, with a total of 40,000 iterations of which 20,000 were discarded as warm-up samples. We assessed model convergence by first computing split-$\hat{R}$ and bulk- and tail effective sample size (ESS) from rank-normalized draws according to Vehtari et al. (2019) and then ensuring ESS > 400 before verifying split-$\hat{R}$ < 1.01. Model parameters were assigned uninformative prior distributions and prior predictive checks were performed following the protocol from Gabry et al. (2019) to verify the use of appropriate prior distributions for all model parameters. We defined varying intercepts at the site level, a structure that was retained for all candidate models to incorporate group-level effects and allow for correlations between samples from the same field site. In addition, model residuals were tested for spatial dependencies using the *DHARMa* package after converting the Bayesian models to DHARma objects with the ´createDHARMa´ function (Hartig, 2018), revealing no significant spatial auto-correlation in residuals across the final models. We selected the optimal model for each hypothesis by comparing model predictive accuracies of candidate models through leave-one-out (LOO) cross validation (Vehtari, Gelman, & Gabry, 2017).

6.2.5.1. Habitat patterns as recorded by BRUVS and eDNA sampling

Since MaxN counts recorded by BRUVS varied 1-2 orders of magnitude depending on the focal species, we fitted separate Generalized Linear Multilevel Models (GLMLMs) for each species to adjust the exponential distributions accordingly. Habitat (a factor with two levels; sand and reef) was the main linear predictor in all BRUVS models. Functional visibility and soak time varied between BRUVS deployments, which we accounted for by adding the logarithm of both these exposure terms as covariates in the GLMLMs. This allows the model to estimate the coefficients of the exposure terms, instead of defining the

terms as an offset (by fixing the coefficient to 1) and implying that recorded species abundance doubles when exposure is twice as high (McElreath, 2016; Zuur & Ieno, 2016). Cod and flounder abundance followed a negative binomial distribution (quadratic parameterization), as substantial overdispersion was diagnosed in the initial Poisson models. Plaice abundance followed a regular Poisson distribution, while wrasse abundance was modelled using a zero-inflated Poisson model to address slight zero inflation in the regular Poisson model (Fig. S6.5).

We examined the proportion of the triplicate qPCR replicates amplified (π_{Amp}) per sample as the response variable for the eDNA data, implying that the response variable was restricted to the values of 0, 0.33, 0.66 and 1. Since a traditional beta regression does not allow inclusion of the boundary values 0 and 1, and we preferred not to transform the y-values, we fitted zero-one-inflated beta (ZOIB) models to allow for outcome values in the [0,1] interval (Liu & Eugenio, 2018). ZOIB models include two distributional parameters in addition to the population mean μ and dispersion parameter ν. The zero-inflation parameter p captures the probability of observing a zero, or $p_{ij} = \Pr(y_{ij} = 0)$ for the jth response on the ith experimental unit, while the conditional-one parameter q refers to the probability of observing a one given the outcome is not zero, or $q_{ij} = \Pr(y_{ij} = 1 | y_{ij} \neq 0)$. Parameters μ, p and q were modelled through a logit-link to ensure values to be constrained between 0 and 1, while dispersion ν was modelled through a log-link to restrict values to the domain of positive real numbers. In contrast to separate species models in the BRUVS analysis, we fitted one pooled model for the eDNA data since the response variable was within the same range for all focal species. The most parsimonious ZOIB model included a Habitat by Species interaction for μ, p and q, varying intercepts per site level for μ, an exposure term (logarithm of sampling volume) on μ only, and an interaction term between Species and standardized SST on parameters p and q.

To obtain a probabilistic interpretation to the BRUVS and eDNA model outputs, we extracted fitted values (i.e. the linear predictor on the response scale; $\hat{Y}$) separately for each of the focal species and computed posterior ratios denoting relative habitat associations of the focal species for each model iteration as:

$$(R_{Habitat})_i = \frac{(\hat{Y}_{Reef})_i}{(\hat{Y}_{Sand})_i} \tag{1}$$

where $(R_{Habitat})_i \in (0, \infty)$, $i = 1, \ldots N$ with N denoting the total number of post warm-up iterations across all chains for each Bayesian model. Hence, a posterior ratio of 1 indicates equal fitted values at reef and sand, whereas a ratio of 0.5 and 2 imply fitted values twice as large at sand and reef habitat, respectively. We then computed the posterior density corresponding to study hypothesis H1 (i.e. $R_{Habitat} > 1$ for cod; $R_{Habitat} < 1$ for flounder etc.) to obtain the associated posterior probabilities.

6.2.5.2. Comparison of detection rates between methods

We converted all MaxN and π_{Amp} values into presence-absence format to obtain comparable values of species detection for both sampling methods. Here, positive eDNA detection was attained if at least one out of three replicate qPCR reactions amplified (hereafter: Amp1). We then used a pooled Bernoulli GLMM to analyze these detection rates, with Method (factor, 2 levels), Species (factor, 4 levels) and their interaction as the main model predictors. We defined a common exposure term for both methods, by setting 1 h soak time (BRUVS) and 1000 mL water sample (eDNA) to an exposure value of 1 and adjusting deviations from these standard exposure values accordingly, prior to log-transforming the covariate. We retained the varying intercepts per site grouping level to allow for within-site correlations induced by our sampling design, while we simultaneously accounted for inherent method-specific correlations by defining varying slopes per sampling method. Similar to equation (1), we defined posterior ratios separately for each focal species to obtain a probabilistic interpretation to between-method differences:

$$(R_{Metho})_i = \frac{(\hat{Y}_{eDNA})_i}{(\hat{Y}_{BRUVS})_i} \tag{2}$$

We then computed the posterior density corresponding to hypothesis (iii) (i.e. $R_{post} > 1$ for all species) to obtain the associated posterior probabilities.

6.2.5.3. Correlation between eDNA signals and MaxN

We investigated potential correlations between eDNA signals and MaxN of focal species by performing a direct comparison of eDNA and the paired BRUVS samples. Specifically, we expressed the eDNA response variable as either π_{Amp} or Amp1 and coupled each eDNA sample with MaxN counts of the two subsequently deployed BRUVS samples to assess whether either eDNA metric correlated with MaxN. Similar to the eDNA analyses above, π_{Amp} was modelled with a ZOIB regression while Amp1 followed a Bernoulli distribution. To account for different exposures in MaxN counts (i.e. varying soak times and functional visibility), we standardized all counts per hour and per m visibility (MaxN · mh^{-1}). We defined separate ZOIB and Bernoulli models for each focal species, with a single population effect of MaxN, allowing us to inspect the amount of variance in eDNA signals explained by species abundance recorded from the BRUVS. In addition to varying intercepts per site ID retained throughout all models, we included a varying MaxN effect per site (i.e. varying slopes) in the ZOIB and Bernoulli model. This additional grouping structure allows for potential site-specific effects of MaxN on eDNA signals, for example due to local differences in vertical mixing at field sites yielding potential variations in eDNA molecule transportation rates.

We made use of the 'hypothesis' function within the *brms* package to obtain a probabilistic interpretation to hypothesis H3, i.e. $\beta_{MaxN} > 0$ for each of the focal species. This function allows for post-hoc testing of contrasts in Bayesian models by computing the posterior probability of a particular hypothesis contrasted against its alternative (Bürkner, 2017). We computed a Bayesian variant of R^2 for the final regression models (Gelman, Goodrich, Gabry, & Vehtari, 2019), yielding posterior distributions of the variance explained by the models for all focal species (Fig. Sx).

6.3. Results

6.3.1. qPCR analysis

Neither the field blank nor laboratory negative controls showed amplification of the target species. Calculated efficiencies and goodness-of-fit (R2) of all analyzed standard dilution series were within the ranges considered appropriate for quantitative qPCR assessment (Bustin et al., 2009) (R2 > 0.99 and efficiency 90%<x<110%; Table S6.2). Estimated eDNA copy numbers were low for all four targeted species. Cod showed the highest estimates ranging from 0-752.4 copies/L, followed by Flounder 0-569.8 copies/L, Wrasse 0-370.0 copies/L and Plaice 0-52.4 copies/L. For the individual reactions, most estimates were below LOQ and in many cases lower than LOD based on the standard dilution series, which represented the lowest standard dilution with positive amplification. In cases where copy number estimates were lower than LOD, estimates still approximated the theoretical minimum detection threshold of one copy per reaction and hence likely represented true positive detections. The measured copy numbers showed positive correlations with the proportion of amplifications (π_{Amp}) for all target species (Fig. S6.2), with posterior probabilities of 99.9% (cod), 99.8% (flounder), 87.6% (plaice) and 99.9% (wrasse) in favor of positive correlations, supporting the use of this parameter as a proxy for concentration.

6.3.2. Habitat associations

6.3.2.1. BRUVS

The BRUVS recordings generally revealed strong habitat patterns in accordance with the expected habitat associations for all four focal species (Fig. 6.2). Cod was on average 22 (CI: 1.1 – 94) times more abundant on reefs relative to sand habitat. The distribution of the posterior ratios for cod indicated a 98% probability of higher cod abundance on reefs compared with sand habitat (Fig. 6.2a; Table 6.2). In contrast, flounder abundance was on average 17 (CI: 1.2 – 81) times higher at sand sites relative to reef sites (Fig. 6.2b), with a 98% posterior probability of detecting more flatfish individuals at sand habitat

(Table 6.2). The recorded habitat pattern for plaice was similar yet slightly less pronounced, with on average 9.4 (CI: 0.5 – 42) times more individuals recorded at sand versus reefs (Fig. 6.2c). The distribution of posterior ratios revealed a 94% probability of observing more plaice at sand habitat relative to reefs (Table 6.2). Finally, BRUVS recorded a reef association for wrasse (Fig. 6.2d), with on average 12 (CI: 0.4 – 58) times higher wrasse abundance compared with sand habitat and a 92% probability of observing more wrasses on reefs (Table 6.2).
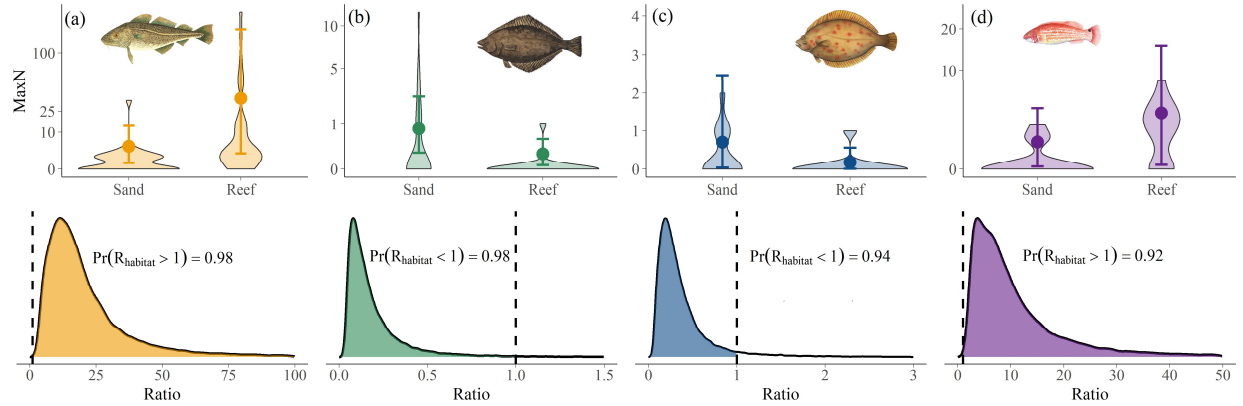

Figure 6.2 – Habitat associations for the focal species as recorded by BRUVS. The violin plots show the raw video data, with the width of shaded areas corresponding to the proportion of data represented at each MaxN count. The large circles with error bars denote the posterior means and 95% credible interval (CI), respectively. Note that some of the y-axes of the upper plots are root-transformed to improve visualization. The four lower plots show the distributions for the posterior ratios, computed according to Eqn. 1. The black vertical dashed line stands at a ratio of 1, implying equal species abundance between the two habitats, while probabilities of observing expected habitat associations are included next to the distribution curves. The four focal species include a) Atlantic cod (*Gadus morhua*); b) European flounder (*Platichthys flesus*); c) European plaice (*Pleuronectes platessa*) and d) goldsinny wrasse (*Ctenolabrus rupestris*). Species drawings by Elisenda Casabona Balcells.

6.3.2.2. eDNA

The proportion of qPCR replicates that amplified (π_{Amp}) was generally high for cod irrespective of the sampled habitat (Fig. 6.3a). We found only a 44% probability in support of hypothesis H1 for cod (i.e. higher π_{Amp} at reefs; Table 6.2), with an average posterior ratio of 0.99 (CI: 0.74 – 1.30) indicating similar amplification rates of cod eDNA across habitats. The eDNA signals for flounder revealed a habitat association in accordance with BRUVS sampling, with a 97% probability of more replicates amplifying at sand habitat (Fig. 6.3b; Table 6.2). On average, π_{Amp} for flounder was 1.88 (CI: 1.00 – 3.48) times higher at sand relative to reef habitat. The detection rate for plaice was generally the lowest of the four focal species in our study (Fig. 6.3c) and provided limited support for a sand habitat association. Posterior

ratios indicated a 67% probability of a higher π_{Amp} at sand habitat compared with reefs, with on average 1.32 (CI: 0.54 – 2.84) times more replicates amplifying at sand habitat. In contrast, eDNA sampling of wrasse confirmed the reef association recorded by BRUVS, with 2.13 (CI: 0.98 – 4.44) times higher π_{Amp} at reefs and a 97% probability of more replicates amplifying relative to sand sites (Fig. 6.3d). We additionally found some species-specific effects of SST on ZOIB distributional parameters p and q. Specifically, SST had a positive effect on p for plaice (i.e. higher temperatures increased the probability of 0/3 amplifications) and a positive effect on q for wrasse (i.e. high temperatures increased the probability of 3/3 amplifications).
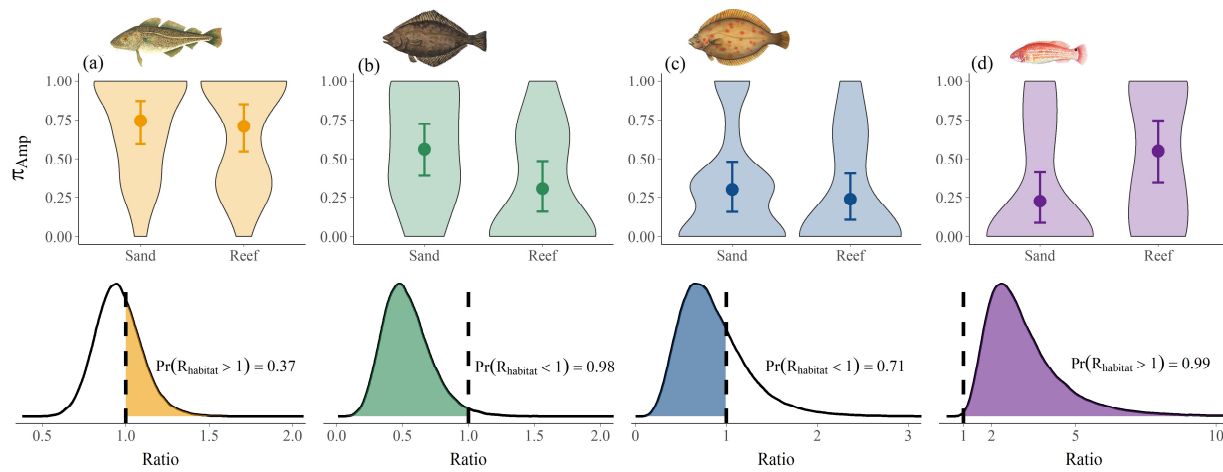

Figure 6.3 – Habitat associations for the focal species as recorded by eDNA monitoring. The upper row of violin plots represent the raw amplification signals, with the width of the shaded areas corresponding to proportion of qPCR replicates amplifying (3 replicates per eDNA sample; possible proportions include 0, 0.33, 0.66 or 1). The large circles and error bars denote the posterior mean and 95% credible interval (CI) from the zero-one-inflated beta (ZOIB) model, respectively. The lower row of density curves show the distributions of the posterior ratios similar to Fig. 6.2, with probabilities of observing expected habitat associations included next to the curves. The four focal species include (a) Atlantic cod (*Gadus morhua*); (b) European flounder (*Platichthys flesus*); (c) European plaice (*Pleuronectes platessa*) and (d) goldsinny wrasse (*Ctenolabrus rupestris*). Species drawings by Elisenda Casabona Balcells.

6.3.3. Method comparison

6.3.3.1. Species detection

Differences in species detection rates from our two sampling methods (BRUVS and eDNA) varied between the focal species (Fig. 6.4). Across habitat type, cod presence was detected on average 1.5 (CI:

1.2 – 1.8) times more often in eDNA relative to BRUVS samples, with a 99% probability of a higher cod detection rate using eDNA sampling (Table 6.2). This pattern remained consistent when accounting for

Table 6.2 – Hypothesis table showing the posterior probabilities associated with the study hypotheses for each of the focal species. $R_{Habitat}$ and R_{Method} represent the posterior ratios computed from fitted values of the Bayesian models according to Eqn. 1 (H1) and Eqn. 2 (H2), respectively. β_{MaxN} represents the slope of the relationship between MaxN and eDNA presence-absence (Amp1) or the proportion of replicates amplifying (π_{Amp}) and for which we computed the probability of taking on positive values in hypothesis H3.

* For sand species in hypothesis 1 and 2, probabilities are computed as $1 - \mathrm{Pr}(R_{post} > 1 \mid y)$ as this corresponds to the hypothesis of observing higher abundance at sand habitat (see Eqn. 1).

	Hypothesis	Cod (*G. morhua*)	Flounder (*P. flesus*)	Plaice (*P. platessa*)	Wrasse (*C. rupestris*)
$\mathbf{Pr}(R_{Habitat} > \mathbf{1} \mid \widehat{y}_{MaxN})$	H1	0.98	0.98 *	0.94 *	0.92
$\mathbf{Pr}\left(R_{Habitat} > \mathbf{1} \mid \widehat{y}_{\pi_{Amp}}\right)$	H1	0.44	0.97 *	0.67 *	0.97
$\mathbf{Pr}(R_{Method} > \mathbf{1} \mid y_{pres-abs})$	H2	0.99	1.00	0.99	0.71
$\mathbf{Pr}(\beta_{MaxN} > \mathbf{0} \mid y_{Amp1})$	H3	0.90	0.85	0.53	0.95
$\mathbf{Pr}\left(\beta_{MaxN} > \mathbf{0} \mid \widehat{y}_{\pi_{Amp}}\right)$	H3	0.52	0.72	0.24	0.53

habitat type, with a 99% and 100% probability of detecting cod more frequently using eDNA sampling at reef and sand sites, respectively (Fig. S6.3). A positive detection signal for flounder was on average 4.2 (CI: 2.4 – 7.3) times more likely using eDNA compared with BRUVS sampling. Posterior ratios indicated a 100% probability of higher flounder detection rate using eDNA sampling (Table 6.2), which remained consistent when accounting for habitat type (Fig. S6.3). Similarly, plaice was on average detected 2.3 (CI: 1.3 – 3.8) times more often with eDNA relative to BRUVS sampling, with a 99% probability of higher plaice detection rates across habitat and > 96% within habitat type (Table 6.2; Fig. S6.3). In contrast, we did not find a strong difference in detection rate of wrasse between the two sampling methods across habitat (Fig. 6.4). On average, eDNA sampling was only 1.2 (CI: 0.8 – 1.7) times more likely to result in a positive wrasse detection relative to BRUVS, with a moderate 76% probability of a higher detection rate using eDNA sampling (Table 6.2). Accounting for habitat type revealed that wrasse was more likely to be detected by eDNA compared with BRUVS sampling at sand (85%) than at reef (56%) habitat (Fig. S6.3).

6.3.3.2. Relationships between eDNA signals and MaxN

We did not find strong and consistent relationships between eDNA signals and recorded MaxN counts across the focal species investigated. While there was a 90% probability of a positive relationship between detection of cod eDNA (Amp1) and abundance recorded by BRUVS (Fig. 6.5a; Table 6.2), this
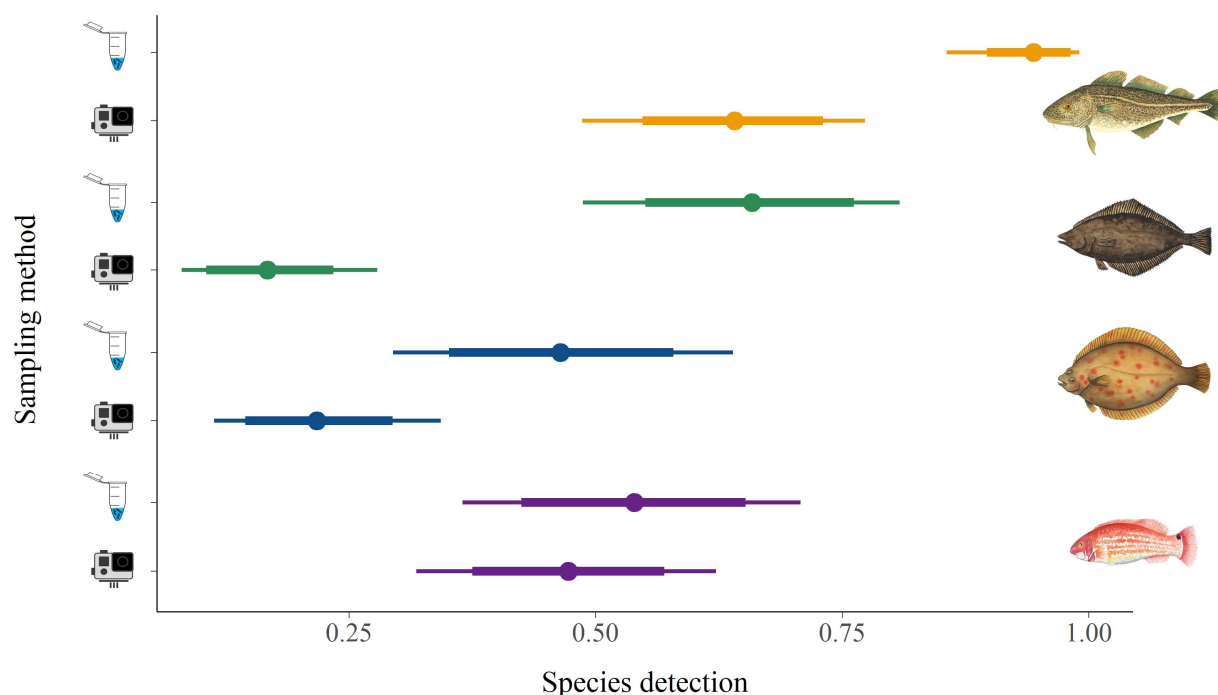

Figure 6.4 – Species detection for each of the focal species achieved by using either video (BRUVS) or eDNA monitoring. Circles denote the posterior mean, thick bars the 80% confidence interval and thin bars 95% confidence interval. Species drawings by Elisenda Casabona Balcells.

relationship had very low explanatory power (median R^2 = 0.05; Fig. Sx). Inspecting the proportion of amplifications (π_{Amp}) as a function of MaxN did not reveal any correlation (Fig. 6.6a), with a probability of 52% of a positive relationship and comparable low explanatory power (R^2 = 0.03). Detection of flounder eDNA had a moderate 85% probability of being positively correlated to flounder MaxN, yet the relationship was substantially stronger relative to cod detections (R^2 = 0.20; Fig. 6.5b). However, these trends disappeared when expressing the eDNA signals as π_{Amp}, with only a 72% probability of a positive relationship and very low explanatory power (R^2 < 0.01; Fig. 6.6b). No evidence was found for a positive relationship between plaice eDNA and MaxN for either type of eDNA response variable (Fig. 6.5c; Fig 6.6c). The probability of a positive correlation was 53% for Amp1 and 24% for π_{Amp}, respectively, and the explanatory power was low for both regression models (R^2 < 0.04). Finally, detections of wrasse eDNA

provided the strongest evidence for a correlation out of all focal species (Fig. 6.5d), showing a 95% probability of a positive relationship with MaxN, which explained considerable amount of the variation in eDNA data (R^2 = 0.16). This trend disappeared however when inspecting the relationship for π_{Amp}, with a 53% probability of a positive relationship (Fig. 6.6d) and little of the variance ($R^2 < 0.01$) explained by wrasse abundance observed on videos.
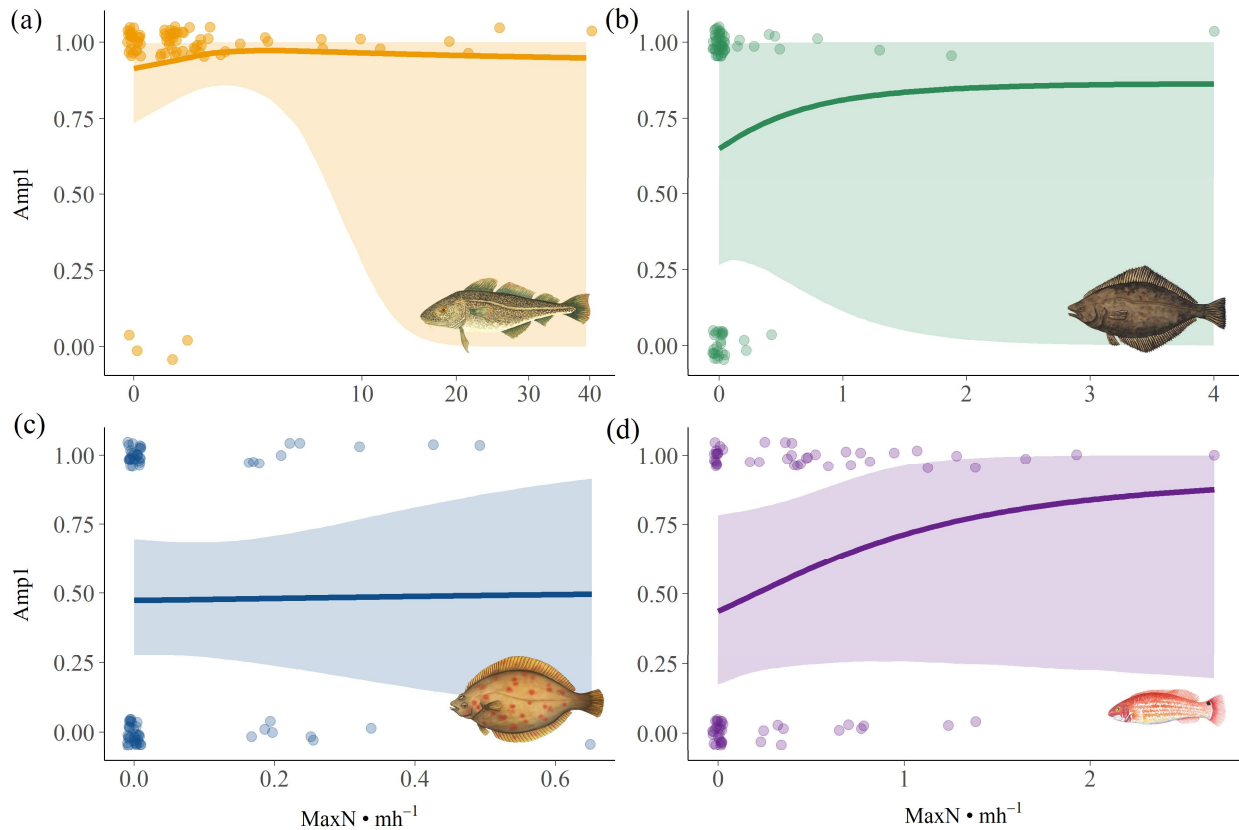
Figure 6.5 – Relationships between eDNA detection rates and relative fish abundance recorded by BRUVS for each of the focal species. Positive eDNA detection is attained when at least 1 out of 3 qPCR replicates amplifies (Amp1). Solid lines and shaded areas represent the posterior means and 95% credible intervals from Bayesian models, respectively, while jittered transparent circles show the raw observations. The MaxN metric was standardized (per meter and hour) to account for variations in functional visibility and soaktime between deployments. Note that the x-axis is root transformed in (a) to improve visualization of smaller MaxN values. Relationships are shown for (a) Atlantic cod (*Gadus morhua*); (b) European flounder (*Platichthys flesus*); (c) European plaice (*Pleuronectes platessa*) and (d) goldsinny wrasse (*Ctenolabrus rupestris*). Species drawings by Elisenda Casabona Balcells.

6.4. Discussion

In this study, we performed a direct comparison between BRUVS and eDNA monitoring of four coastal marine species in the western Baltic Sea (WBS). Despite very low copy numbers of target species in the eDNA samples, our comparative analysis showed that eDNA was capable of detecting fine-scale habitat associations for two species in agreement with BRUVS. We explored a semi-quantitative approach by expressing eDNA signals as the proportion of qPCR replicates amplifying, which revealed higher proportions at sand sites for a sedentary benthic predator (flounder) while reef sites yielded more amplifications for a highly site-attached reef fish (wrasse). No association was detected from eDNA traces of highly mobile cod, showing ubiquitous amplification signals across the bay in contrast to a strong reef-association documented by BRUVS. Detection of plaice was generally low for both methods, yet the use of a relative abundance metric (MaxN) in BRUVS still revealed a sand-association contrary to no difference across habitat for eDNA. Conversely, eDNA sampling yielded higher detection rates for cod, flounder and plaice relative to BRUVS, while wrasse was equally detected between the two sampling methods. This result indicates there is potential for eDNA to outperform BRUVS in terms of species detection, even for mobile and conspicuous predatory fishes like cod for which the bait attractant in BRUVS facilitates efficient monitoring. Finally, eDNA detection rates showed a moderate to high probability of a positive correlation with MaxN counts for cod, flounder and wrasse. However, these trends disappeared when expressing eDNA signals as proportions of replicate amplifications, indicating there was no direct relationships between relative abundance of target species recorded on videos and the number of amplifications in the paired eDNA samples. Our study highlights some of the opportunities and limitations associated with BRUVS and eDNA monitoring. Given that the low observed DNA concentrations precluded a fully quantitative treatment of eDNA data, it is apparent that the higher resolution provided by the MaxN metric in BRUVS data yielded more informative inference on relative species occurrence in our study. Still, we demonstrate that fine-scale habitat associations can be detected for sedentary species at low concentrations by inspecting amplification signals, a finding which given the superior species detection rates obtained from eDNA sampling suggests that the combined use of both methods could add valuable knowledge to monitoring programs and thus better inform marine conservation efforts.

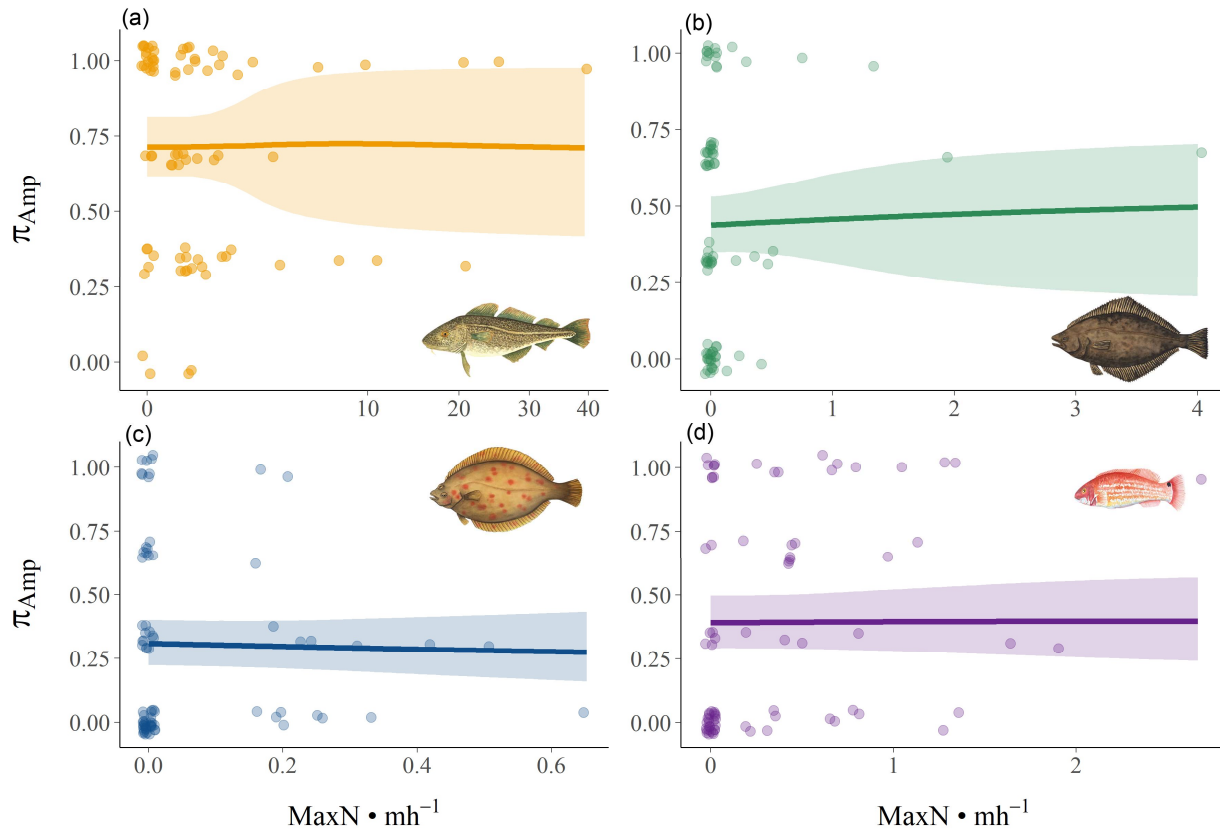Figure 6.6 – Relationships between the proportion of qPCR replicates amplifying (π_{Amp}) and relative fish abundance recorded by BRUVS for each of the focal species. See Figure 6.5 caption for further explanation. Species drawings by Elisenda Casabona Balcells.

6.4.1. Habitat patterns and species mobility

The four focal species in our study were selected based on documented habitat use (i.e. either reef or sand-associated) and varying degrees of mobility from sedentary to highly mobile. Both BRUVS and eDNA monitoring revealed strong habitat associations for flounder and wrasse, while associations for cod and plaice were only apparent from BRUVS samples (Table 6.2). We suggest that these results at least partly reflect the ecology and mobility of our target species. Atlantic cod is an important apex predator in the Baltic Sea and multiple studies have previously highlighted the importance of hard bottom structures for survival and growth of cod (Gotceitas & Brown, 1993; Lindholm, Auster, & Kaufman, 1999; Schwartzbach, Behrens, & Svendsen, 2020). However, habitat use by this mobile demersal fish has been shown to be highly dynamic and to vary with diel and seasonal movement patterns in response to changes in food availability, sea surface temperature and thermal stratification (Freitas, Olsen, Knutsen, Albretsen, & Moland, 2016; Funk, Krumme, Temming, & Möllmann, 2020). Therefore, despite a documented preference for hard substrates of high structural complexity in older juveniles and adults, cod

individuals exhibit active movement patterns within the larger seascape and still make use of soft-bottom areas to some degree (Cote, Moulton, Frampton, Scruton, & McKinley, 2004). For example, cod may use soft-bottom areas to forage on shore crab (*Carcinus maenas*), which is one of the main prey items for adult cod in the SW Baltic Sea (Funk, Frelat, Möllmann, Temming, & Krumme, 2021), or to simply move between different reef patches within a coastal mosaic of various habitat types. Still, the use of MaxN as a relative abundance metric derived from BRUVS data enabled us to discern a strong reef association for cod, with a 98% probability of higher cod abundance at reef sites. In contrast, eDNA sampling did not detect a reef association for cod and indicated similar proportions of qPCR replicates amplified at reef and sand sites. This apparent discrepancy between the two sampling methods might be partly explained by the relatively low resolution obtained from qualitative (or semi-quantitative) eDNA data combined with the high mobility of cod. In particular, transient visits to soft-bottom habitats by cod may have resulted in substantial shedding of eDNA particles and subsequent high detection rates of cod eDNA across habitats. As we only used three qPCR replicates in this study, it could be possible that this provided limited potential for discerning habitat patterns and that a higher number of replicates (e.g. 6 to 12 replicates; Furlan et al., 2019) might instead have produced detectable differences in amplifications between reef and sand habitats. In addition, the eDNA traces from cod may represent a substantially wider temporal coverage compared with the 1-2h snapshot provided by BRUVS, given documented persistence time of eDNA molecules within marine environments (though strongly dependent on species and environmental conditions) in the order of hours to days (Thomsen et al., 2012; Weltz et al., 2017). This implies that observed eDNA signals of cod could represent diel variations in movement patterns of cod that we were unable to observe using daytime BRUVS deployments. Future studies employing BRUVS and/or eDNA should consider using a higher number of qPCR replicates and comparing day and nighttime BRUVS recordings, to shed more light on inter-method discrepancies in documenting habitat use by mobile predators as observed in this study.

Flounder and plaice are sedentary benthic predators that mainly feed on tube-building polychaetes, small crustaceans and mollusks (De Groot, 1971; Doornbos & Twisk, 1984). Although both species are known to undertake ontogenetic migrations in response to predation risk and temperature changes (Gibson, Robb, Wennhage, & Burrows, 2002; Amorim, Ramos, Elliott, & Bordalo, 2018), they generally exhibit a sedentary lifestyle with substantial periods of immobility typical of cryptic benthic predators (Gibson, Stoner, & Clifford, 2005). For flounder, both BRUVS and eDNA monitoring documented a strong association with sand habitat, with probabilities of 98% and 97% respectively of higher flounder detections relative to reef sites. These results provide evidence of localized eDNA signals for sedentary flounder within our coastal study area characterized by a mosaic of different seabed types. Despite hard-bottom substrate being in close proximity to our sand sites and vice versa (few 100 m's away, Fig. 6.1), we did not find a homogenous eDNA signal for flounder across the bay implying that either flounder DNA was not transported very far or rapidly became too diluted for detection with increasing distance from the source. Average current velocities across our study sites ranged from 0.9 to

4.8 cm/s, (Fig. S6.1) and combined with an estimated time of 6.7 days until flounder eDNA degrades beyond the detection threshold (Thomsen et al., 2012), we roughly estimate that DNA molecules could have been transported between 5.2 and 27.8 km away from the source on average. We therefore hypothesize that rapid dilution of flounder eDNA in the marine environment was the most important factor contributing to the localized signals found in this study. Since flounder is mostly confined to bottom habitat during the day, our results furthermore indicate that the surface water samples we collected for filtration nonetheless contained sufficient particles of flounder DNA to detect an association of flounder with sand habitats. Despite the expected strengthening of thermal stratification in the WBS across the study period (Funk et al., 2020), vertical mixing or coastal upwelling may have induced sufficient transportation of DNA material from the seabed (at ~ 5-10 m depth across sites) to the surface water layer to be detected in our eDNA samples. In contrast, eDNA traces for plaice were generally low in this study (0-52.4 copies/L) and we did not detect any habitat association for plaice using eDNA monitoring. One possible explanation for the low copy numbers for plaice could be that this species is relatively less common in the WBS than the confamilial flounder, which is widely distributed across most of the Baltic Sea (Nissling, Westin, & Hjerne, 2002). However, BRUVS recorded roughly equal occurrences of flounder and plaice across our study sites, both in terms of relative abundance (MaxN; Fig. 6.2) and species detection (presence-absence; Fig. S6.3). Both flatfish species were highly attracted to the bait and often observed to be actively feeding for prolonged periods once attracted into the camera's field of view. This bait plume effect in BRUVS may therefore have provided a significant advantage over eDNA monitoring, by attracting individuals in the vicinity to the camera and thereby increase the statistical power to make inferences on habitat associations for plaice. In case plaice individuals were highly dispersed across the study sites, the eDNA particles shed by these individuals may have been too diluted to produce detectable signals in seawater samples. An interesting future avenue for studies comparing BRUVS and eDNA monitoring could therefore be to assess whether eDNA detection of species increases post-BRUV deployment due to the bait plume effect, although bait contamination should be a critical consideration and could potentially hinder eDNA inference from water samples taken after BRUVS deployment (Jeunen et al., 2020).

The goldsinny wrasse is a small-bodied mesopredator that plays an important trophic role within reef ecosystems by serving as a prey item for large reef predators such as cod (Funk et al., 2021), while indirectly shaping macroalgal communities through predation on mesograzers such as amphipods and isopods (Östman et al., 2016). Goldsinny wrasses are actively swimming reef fishes, yet they are highly site attached with males defending territories with a mean area of 1.4 m^2 and no larger than 2 m^2 (Hilldén, 1981). Individuals are highly associated with reef structures such as holes and crevices, and are rarely observed far away from suitable refuges (Sayer, Gibson, & Atkinson, 1993). Our results confirm a strong reef-association in goldsinny wrasse, as both BRUVS and eDNA monitoring yielded high probabilities (92% and 97% respectively) of higher wrasse detections at reef sites. We note that eDNA results for wrasse are likely reflective of their highly site-attached behavior, as eDNA traces appeared to be localized instead of homogeneous across the relatively small study area. Traditionally, goldsinny wrasse has been

of little economic value (Sayer et al., 1993), yet commercial interest increased rapidly in the early 90s after discovery of the potential use of wrasse as a cleaner fish to counteract lice infestations within the salmon aquaculture industry (Bjordal, 1991). The development of a wrasse fishery was initially found to have negligible impacts on wrasse populations in Norway (Gjøsæter, 2002), yet there has been increasing concern about the sustainability of the fishery as catch rates have increased significantly over the past decades (Skiftesvik et al., 2014; Blanco Gonzalez & de Boer, 2017). Given the ongoing need to monitor the effects of intensifying wrasse fisheries in Northern Europe, our newly developed qPCR assay for goldsinny wrasse could be used to facilitate rapid and low-cost detection of wrasse eDNA in marine environments. We suggest that a combined use of eDNA sampling and video surveillance is a promising, non-invasive monitoring strategy to document spatiotemporal distributions of wrasse in coastal waters.

The use of ZOIB models in our study revealed some species-specific effects of temperature on the proportion of qPCR replicates amplifying. Specifically, water temperature had a positive effect on the zero-inflation parameter (p) for plaice, implying that the probability of observing 0/3 amplifications increased at higher temperatures. One explanation for this effect could be that plaice were less abundant at the study sites in summer (late June) compared with spring (late April), potentially moving to deeper waters to avoid the higher water temperatures and thereby causing a higher proportion of samples to yield zero amplifications. Alternatively, eDNA particles are known to degrade more rapidly when exposed to high temperatures (Strickler et al., 2015; Hansen et al., 2018), which could also explain the observed temperature effect for plaice. No similar effects of temperate were found; however for the remaining three focal species (95% CI's included zero), making it more likely that the effect in plaice was due to a temperature-driven shift in the spatial distribution of plaice within the study area, a trend at least partly confirmed by BRUVS observations (Fig. S6.4). In contrast, the conditional-one parameter (q) was positively affected by temperature for wrasse (Fig. Sx), implying a higher probability of 3/3 amplifications at higher water temperatures. Previous studies conducted within controlled environments have reported a positive effect of water temperature on eDNA concentrations (Lacoursière-Roussel et al., 2016) and shedding rates (Jo, Murakami, Yamamoto, Masuda, & Minamoto, 2019), largely attributed to an increased metabolic rate of the study organisms under increased temperatures. However, it currently remains challenging to disentangle the diverse ways in which temperature may affect eDNA concentrations in marine systems and the contrasting effects of increased shedding (i.e. production) or decay combined with seasonal changes in species distributions and activity levels likely yield varying effects across study systems and organisms (Rourke et al., 2021). Still, our study exemplifies how the use of ZOIB models can provide further species-specific information on factors influencing the absence and/or complete amplification of qPCR replicates in marine environments with low DNA concentrations.

6.4.2. High sensitivity in eDNA but lack of correlation between amplifications and MaxN counts

Our inter-method comparison of species detection rates revealed that eDNA sampling yielded higher detection rates for three out of four focal species in the study area (Fig. 6.4). These results add to the growing body of evidence indicating superior species detection using eDNA monitoring relative to other monitoring techniques (Schmelzle & Kinziger, 2016; Hinlo, Furlan, Suitor, & Gleeson, 2017; Weldon et al., 2020; Valdivia-Carrillo, Rocha-Olivares, Reyes-Bonilla, Domínguez-Contreras, & Munguia-Vega, 2021). Contrary to our expectation, eDNA sampling was more efficient in detecting Atlantic cod, a highly conspicuous predatory fish that is easily identified using BRUVS because of its relatively large size and active feeding behavior in response to the bait. Still, high eDNA detection of cod was attained at sand sites (Fig. 6.3; Fig. S6.3) where cod were observed in fewer numbers by BRUVS and seemingly less attracted to the bait. This suggests that some cod individuals may have been missed by BRUVS if they were present at the sand site but remained outside the camera's field of view. Interestingly, eDNA and BRUVS sampling showed comparable detection rates for goldsinny wrasse (Fig. 6.4). Similar to cod, goldsinny wrasse is a conspicuous reef fish and therefore easily identified and counted in BRUVS recordings. While wrasses were generally not responding directly to the bait in our study, they seemed to be attracted to overall increased activity levels of feeding organisms as has also been reported for non-piscivorous fishes in other regions (Harvey et al., 2007; Watson et al., 2010). Combined, the strong site fidelity in wrasse, the equal detection rates between BRUVS and eDNA, and the clear reef-association documented by both techniques indicate that wrasse eDNA traces were localized within the reef sites and either not transported far away from the source or too diluted to be detected at the nearby sand sites. This would suggest that both methods are highly suitable for monitoring of conspicuous marine species with small homing ranges, yet BRUVS provided the additional advantage of a relative abundance metric whereas the low DNA concentrations in our study area precluded a quantitative analysis from the eDNA data.

Although we found some evidence in our results of higher eDNA detection rates when the relative abundance (MaxN) of focal species was high (Fig. 6.5), there was a general lack of quantitative relationships between the proportion of eDNA amplifications and MaxN for all focal species (Table 6.2; Fig. 6.6). There have been a number of studies reporting a similar lack of correlation with abundance metrics from traditional methods (Hinlo, Lintermans, Gleeson, Broadhurst, & Furlan, 2018; Deutschmann, Müller, Hollert, & Brinkmann, 2019; Knudsen et al., 2019), which can be expected as correlations weaken significantly in dynamic natural systems relative to controlled environments (Yates et al., 2019). Still, a recent literature review revealed that up to 90% of published comparative studies have documented a positive relationship between eDNA signals and fish abundance or biomass recorded by traditional methods (Rourke et al., 2021). While a publication bias against studies reporting no effect is likely affecting this finding (Rourke et al., 2021), the overwhelming trend in positive correlations reported across study systems and organisms certainly provides a promising outlook in terms of using eDNA as a

complementary tool for rapidly monitoring spatiotemporal distributions of aquatic species. The comparative assessment of our study included two methods with undefined spatial coverage and we suggest that the lack of correlations may at least be partly explained by an expected difference in sampled area between the two methods. The temporal 'snapshot' provided by both methods is also likely to be different (Jeunen et al., 2020), since BRUVS recorded for 1-2 hours and continuously attracted individuals that may not have been included in the eDNA 'snapshot' taken prior to BRUVS deployments. We also cannot completely rule out the effects from small-scale and recreational fishing activities frequently observed near our study sites, since the presence of dead individuals in fishing nets may have 'contaminated' our eDNA samples and further obscured relationships with MaxN (Hansen et al., 2018). Furthermore, it is possible that the use of π_{Amp} as the response variable in the eDNA analysis masked potential correlations with MaxN that would otherwise have been apparent when using traditional eDNA concentrations. However, copy numbers consistently below the LOQ threshold in this study prevented us from directly using eDNA concentrations (Klymus et al., 2020), whereas the strong correlations with π_{Amp} for all focal species (Fig. S6.2) justified the use of π_{Amp} as a proxy for concentrations instead. We suggest that similar future studies conducted within systems of low concentrations should increase their sampling volume (i.e. > 1 L of seawater) and use a larger number of qPCR replicates, which could potentially yield additional ecological insights. Here, the use of three qPCR replicates implied that π_{Amp} was limited to only four discrete values, whereas 6-12 replicates would have increased the resolution of the eDNA data and could thereby benefit statistical inference. Finally, we note that the challenge of identifying organisms with cryptic appearance in BRUVS is likely to have contributed to the lack of correlations for the two flatfish species in our study. Specifically, flatfish individuals were assigned to family level instead of species level in 45% of observations, which mostly represented cases in which individuals were clearly right-eyed (family *Pleuronectidae*) but subtle features distinguishing the different species were not visible on the videos. These observations had to be excluded from the analysis since they could not be confidently assigned to either flounder or plaice and this exclusion could thus have significantly affected correlations with eDNA signals. However, given the similarly weak relationships for conspicuous cod and wrasse (assigned to family level on 9.9% and 5.7% of BRUVS observations, respectively), it is safe to argue that the lack of correlations cannot be solely attributed to the challenge of identifying cryptic species using BRUVS. This challenge does, however, highlight the benefit of using eDNA monitoring in tandem with other non-invasive methods to improve the taxonomic precision of monitoring efforts.

6.4.3. Limitations and future prospects

The use of mono-cameras in our study precluded accurate body size measurements of individuals in the BRUVS recordings, which subsequently restricted us to only recording the relative abundance (i.e. MaxN) of the focal species instead of exploring additional metrics such as fish length and biomass distributions. However, stereo-BRUVS are rapidly becoming the global standard in underwater

video monitoring efforts (Langlois et al., 2020), with synchronized and calibrated cameras allowing for a depth perspective to video images and accurate size estimates of marine organisms. It could therefore be interesting for future studies to pair eDNA sampling with stereo-video systems to investigate potential relationships of eDNA signals with recorded fish biomass, as well as to assess variations in measured eDNA concentrations for different size classes (and associated surface area; Stoeckle et al., 2021) of target species (Maruyama et al., 2014). While both eDNA and BRUVS techniques have their own intrinsic biases and shortcomings with respect to sampling marine communities, these non-invasive methods are still actively improved and will likely benefit from further technological advances in the near future. For instance, BRUVS analyses currently involve the processing of large amounts of video material by trained observers with taxonomic expertise, which can be both costly and time-consuming. The heavy reliance on manual annotation and taxonomic expertise might in the near future be completely alleviated by machine learning algorithms that provide rapid counts and more accurate identifications of focal taxa (Marini et al., 2018; Lopez-Vazquez et al., 2020). However, substantial barriers to common use of algorithms in video surveys remain, including a need for huge amounts of labelled training data, high computational demands associated with currently used algorithms and the limited expertise in machine learning techniques generally available within ecological study programs (Siddiqui et al., 2018; Dujon & Schofield, 2019). Arguably, one of the most promising ongoing developments within the field of eDNA monitoring is that of autonomous real-time processing and measuring of eDNA signals using an environmental sample processor (ESP; Ussler et al., 2013; Hansen et al., 2020). The ESP functions as a submersible DNA laboratory and enables automated *in situ* collection, filtration, extraction and analysis of DNA samples while transferring results within hours to users based on land (Hansen et al., 2020). This technology has the potential to revolutionize long-term sampling of a wide range of habitats, in particular harsh offshore environments, by circumventing some of the limitations regarding safety and logistics commonly associated with employing other non-invasive methods (Delefosse et al., 2020). We note that herein lie further opportunities for the co-development of both methods, as autonomous eDNA samplers could potentially be equipped with video cameras to record the surrounding environment at preset time intervals across extended periods. The current development of a mobile, self-propelling sampling unit (Pargett et al., 2015) could even increase the spatial resolution of such surveys, allowing for adjustments of the locality at which samples are taken to cover large parts within a study area of interest. Concurrently, sampling of entire communities using metabarcoding might soon be feasible on-site through small portable instruments (Zaaijer et al., 2017; Hansen et al., 2018) that could facilitate in-tandem use with traditional survey methods during field expeditions for rapid assessment of local community structures. We note that prior to potentially realizing any of the promising developments above, eDNA and video sampling can already be combined with relative ease in the field by bringing low-cost enclosed Sterivex filters (Spens et al., 2017) on field expeditions to filter water samples prior to camera deployments. The filtered samples can afterwards be stored at freezing temperature for later use, in case further eDNA analysis is not immediately feasible. In this study, it took approximately 20 minutes to filter a 1L water

sample using relatively simple and low-cost sampling equipment, which in our view is a minimal effort given the superior sensitivity offered by eDNA to detect target species. The integrated use of both survey methods would add substantial scientific information in terms of species occurrences and distributions relative to using either method in isolation (Stat et al., 2019; Mercaldo-Allen et al., 2021) and would therefore more effectively guide management actions aimed at conserving coastal marine ecosystems.

6.5. Conclusion

Adaptive management strategies to mitigate anthropogenic impacts on coastal ecosystems demand accurate scientific information on biodiversity in terms of spatiotemporal distributions of inhabitant species. Our study has demonstrated that eDNA sampling can detect habitat associations for sedentary species within a small coastal area (~ 20 km^2) characterized by a mosaic of different seabed types. These results add to the growing evidence of localized eDNA signals within coastal marine ecosystems and simultaneously suggest that the ability of eDNA monitoring to document SHAs is to some extent dependent on the mobility and ecology of focal taxa. For highly mobile species and species detected at very low concentrations, our results indicate that monitoring studies might benefit from supplementing eDNA with BRUVS sampling to yield more informative inference through a higher resolution offered by the MaxN metric and the use of bait attractants to overcome the challenge of monitoring widely dispersed organisms within field sites. Conversely, eDNA sampling outperformed BRUVS in overall detection rates of three out of four species, with equal detection for the remaining species, and therefore has much to offer in terms of superior sensitivity in detecting target species, even for piscivorous fishes that are well represented in BRUVS recordings (e.g. cod). We recommend that future eDNA studies conducted within systems of low DNA concentrations further explore the use of replicate amplifications (i.e. π_{Amp}) as a response variable and how this metric relates to species abundances derived from conventional methods. The lack of any relationship between π_{Amp} and MaxN in this study suggests limited scope for directly relating eDNA signals to individuals recorded by paired BRUVS, yet the potential of inferring habitat associations from amplification signals will likely benefit monitoring and conservation efforts of aquatic systems with low species densities.

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Supplementary Information

S1 - eDNA extraction protocol – modified from Spens et al. (2017)

1. Carefully wipe the outer surfaces of the filter capsules with 5% bleach using clean tissue paper. Dry and wipe with 70% Ethanol using tissue paper.

2. Make a premix of Lysis working solution by adding 720 µL ATL buffer and 80 µL proteinase K per sample. Carefully add 800µl Lysis working solution to the filter by using a 1,000µL pipet and sterile filter tips. Pipet the solution between the outside of the filter and the capsule walls. Seal with parafilm. Handshake vigorously for a few seconds.

3. Incubate, while rotating, at 56°C for 2 hours.

4. Handshake SX filter capsules vigorously 5 times.

5. Remove ALL the liquid from inlet end of capsule by using a Luer Lock syringe. Measure the volume, transfer to 5 mL LoBind tube. Vortex for a few seconds. Spin down for 2 seconds to seed out excess debris.

6. Add Buffer AL and ice cold molecular grade 99.99% ethanol to the sample in equal volumes. Sample:Buffer:Ethanol = 1:1:1. Note: AL and ethanol can be premixed.

7. Vortex vigorously

8. Pipet the mixture (max 650 µL at a time) into a DNeasy Mini Spin column in a 2 mL collection tube provided in the kit.

9. Spin in micro-centrifuge preferably at 4°C at 6000∗g (8000 rpm for rotor max capacity 24∗1.5-2 mL tubes) 1 min

10. Discard flow-through.

11. Repeat steps 10-12 until all sample is filtered through DNeasy Mini spin column.

12. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at 6000∗g(8,000rpm). Discard flow-through and collection tube.

13. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000∗g(14,000rpm) to dry the DNeasy membrane. Discard flow-through and collection tube. Place spin column in a new collection tube, centrifuge 1 min at 17,000∗g (13,000rpm).

14.   Transfer spin column to a new 1.5 or DNA LoBind tube.

15.   Add 100 μl preheated Buffer AE (70°C) to the membrane.

16.   Incubate at room temperature for 10 min.

17.   Centrifuge for 1 min at 6,000 $*$ g (8,000 rpm)

18.   Discard the spin column.

19.   Store at -20°C or at -80°C.

Table S6.1 - Results of the *in silico* analysis of the cytochrome b gene and of number of mismatches between the selected primers and probe and species related to the target species, goldsinny wrasse (*Ctenolabrus rupestris*).

Species	Number of mismatches (fixed/none fixed)			Included in in vitro test
	CterupR_F705	Cterup_P791	Cterup_R842	
Goldsinny wrasse (*Ctenolabrus rupestris*)	0/0	0/0	0/0	√
Cuckoo wrasse (*Labrus bimaculatus*)	2/0	4/0	2/0	
Mediterranean rainbow wrasse (*Coris julis*)	1/2	3/3	1/5	
Ballan wrasse (*Labrus berggylta*)	4/0	6/0	2/0	√
Bluehead wrasse (*Thalassoma bifaciatum*)	4/0	3/0	5/0	
Corkwing wrasse (*Symphodus melops*)	NA	NA	NA	√

Table S6.2 - Information on goodness-of-fit (R^2) and efficiencies for each qPCR run in the study. LOQ and LOD are also shown in the table. LOD is defined as lowest copy number detected in at least one of reaction of the dilution series. LOQ is defined as the lowest copy number detected in all triplicates in the standard dilution series.

Assay	Plate no*	R^2	Efficiency	LOQ	LOD
Atlantic cod (*Gadus morhua*)	Plate 1	0.997	98.907	40	40
	Plate 2	0.997	104.274	40	4
European flounder (*Platichthys flesus*)**	Plate 1	0.997	96.596	400	400
	Plate 2	0.999	98.907	400	400
European plaice (*Pleuronectes platessa*)	Plate 1	0.997	102.423	4	4
	Plate 2	0.998	95.260	4	4
Goldsinny wrasse (*Ctenolabrus rupestris*)	Plate 1	0.995	99.379	4	4
	Plate 2	0.997	95.010	40	4

* all the samples were analyzed in two qPCR plates for each species.

** based on a standard dilution series with 4E7-4E2 copies, all other assays were based on a standard dilution series with 4E5-4E0 copies
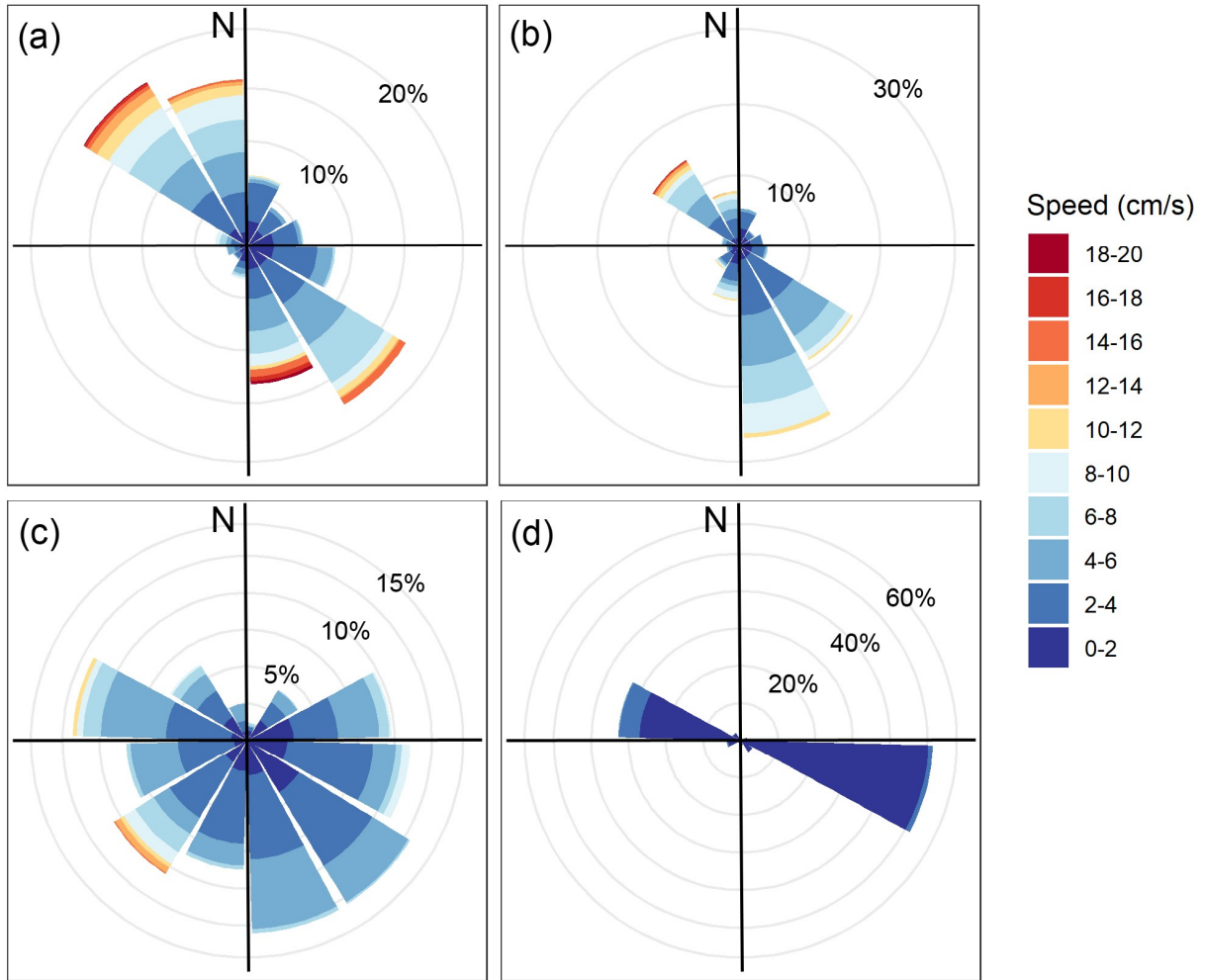
Figure S6.1 – Rose diagrams showing current direction and velocities at the four sites within the study area. The proportions of observed directions and speed categories is indicated next to the circles. Current directions and velocities are shown for Kegnæs Ende (a); Spar Es (b); Vesterhage (c) and Viemose (d). See Fig. 6.1 for the locations of the sites within the bay. Directions indicate where the current is flowing towards. For example, at Viemose (d) water was flowing predominantly towards the east-southeast (ESE; coming from west-northwest [WNW]) and towards WNW (coming from ESE). Data used in plots obtained from the Danish Hydraulic Institute (DHI).
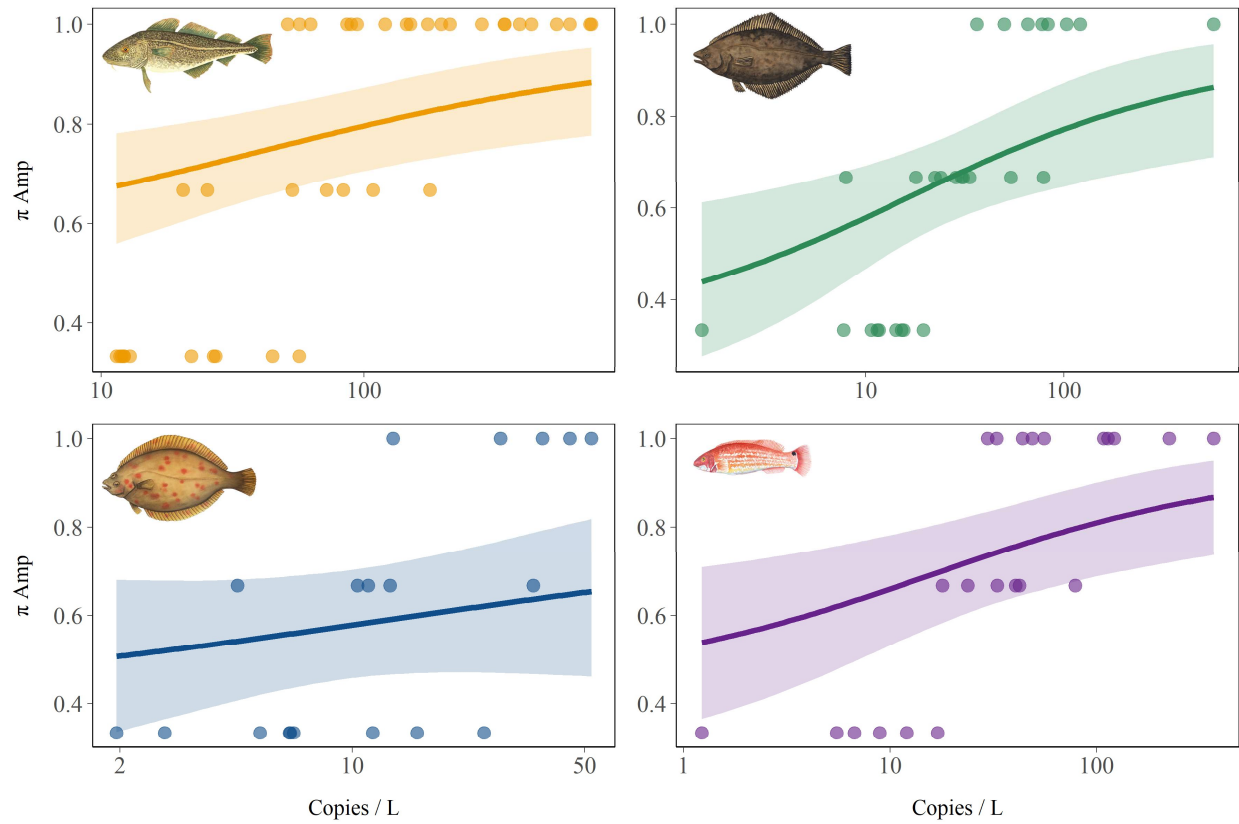
Figure S6.2 – Relationships between measured DNA copy numbers (copies / L) and the proportion of qPCR replicates amplifying within a sample (π_{Amp}) for all focal species. Species drawings by Elisenda Casabona Balcells.
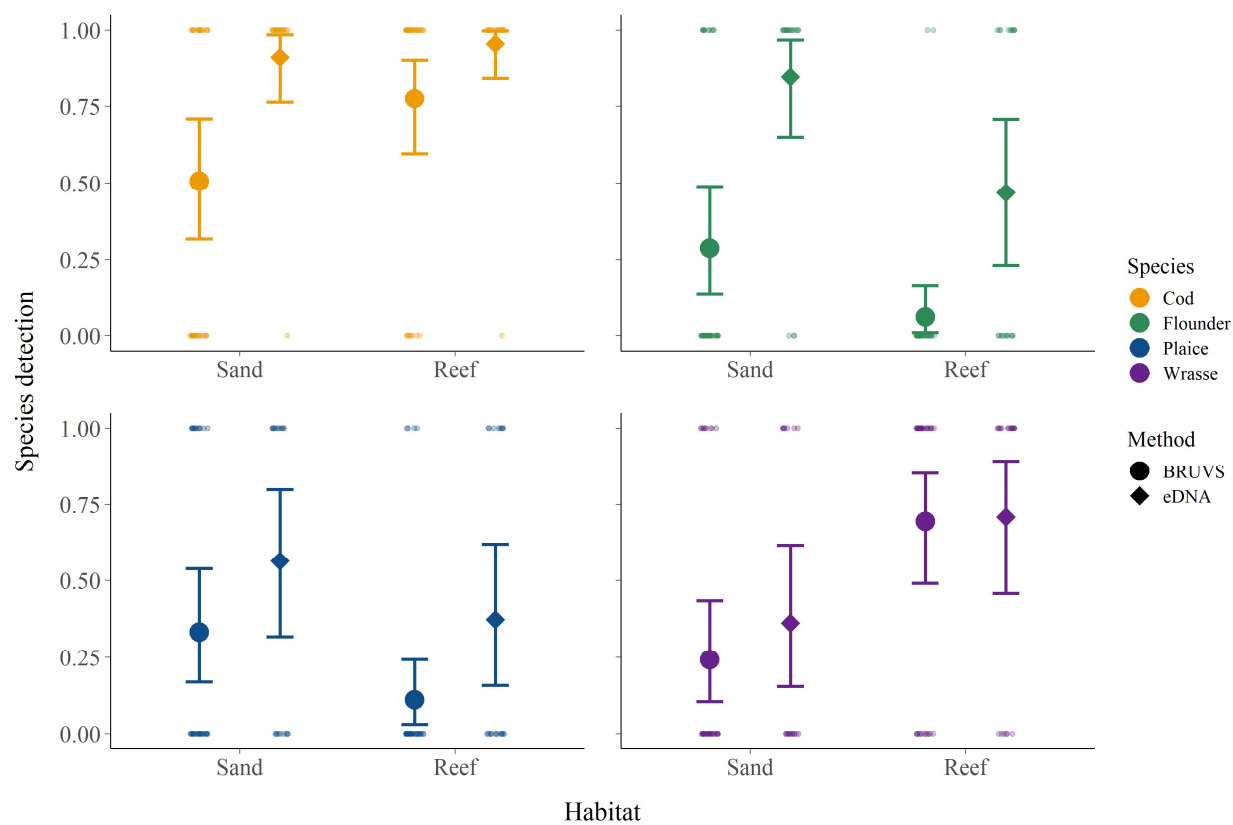
Figure S6.3 – Species detection rates between sand and reef habitats as obtained from BRUVS (circles) and eDNA (diamonds) sampling.
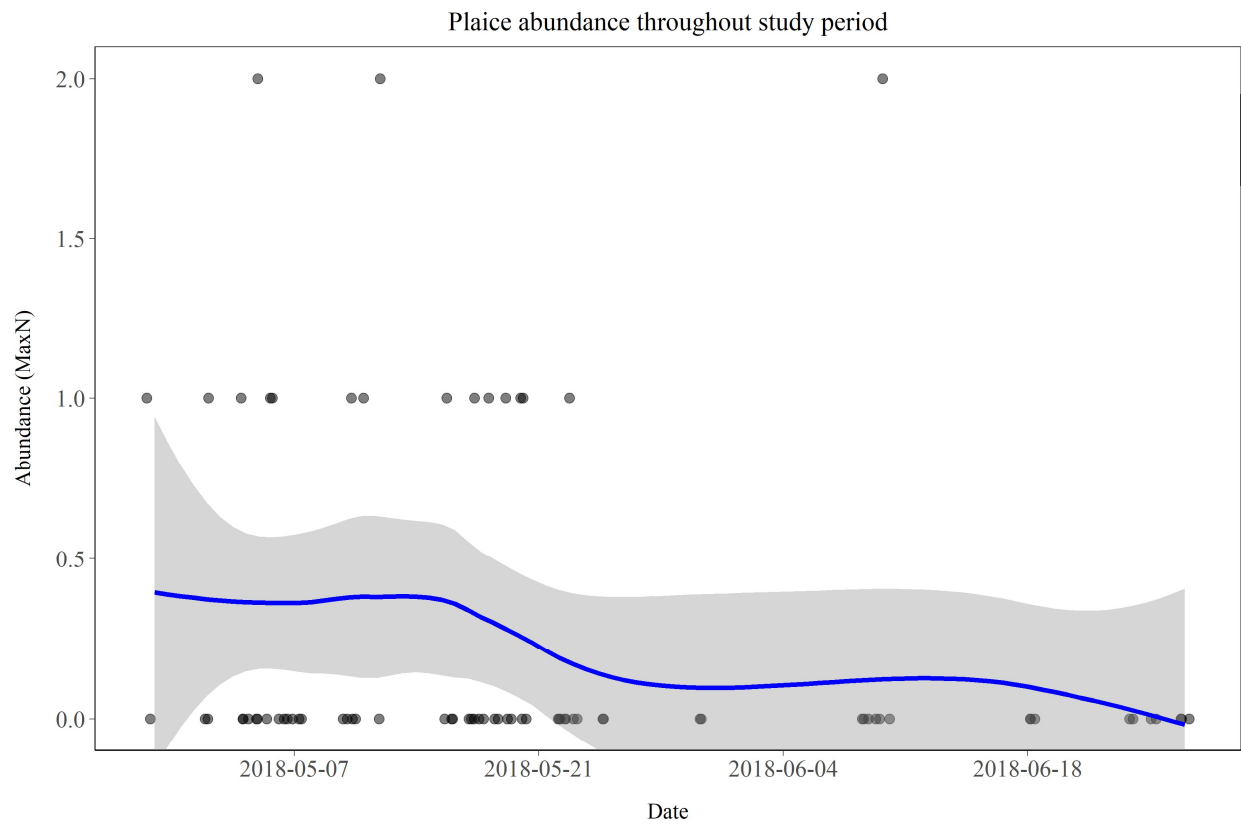
Figure S6.4 – Plaice relative abundance (MaxN) throughout the study period as recorded by BRUVS. Mean (blue line) 95% confidence interval (grey shaded area) obtained from loess smoothing.
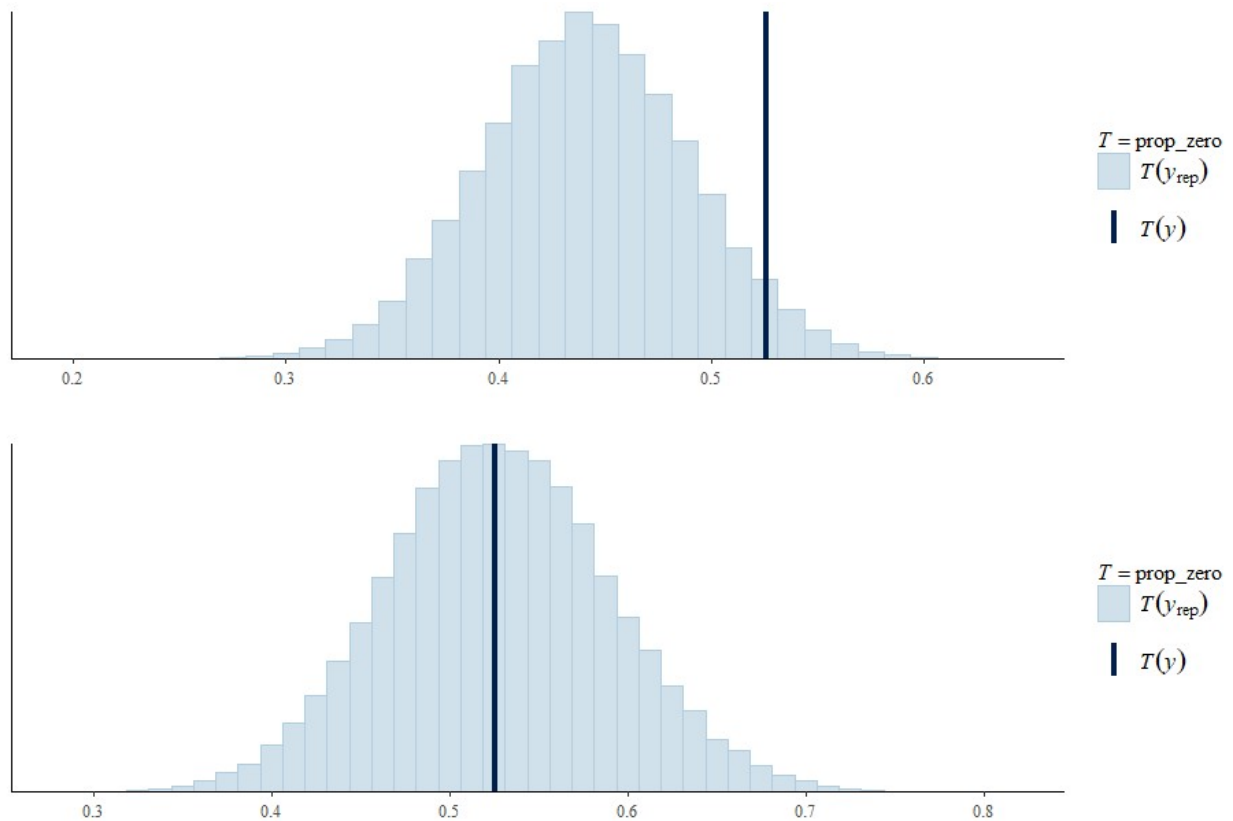
Figure S6.5 – Zero-inflation test based on 40,000 simulations from the posterior predictive distribution from a regular Poisson (upper graph) and zero-inflated Poisson model (lower graph) on goldsinny wrasse abundance. The solid vertical line represents the proportion of zeros in the BRUVS data, while the histogram shows the distribution of zero counts within the simulated datasets. Under a regular Poisson, the number of zeros in the dataset is slightly higher than covered by most simulated datasets (although still within bounds), while adding a zero-inflated structure to the model clearly improves the fit.

Chapter 7

General discussion

The aim of this thesis was to document the effects of two independent reef restoration projects carried out in the western Baltic Sea (WBS), and to assess the potential of various non-invasive monitoring techniques to record relationships between coastal marine taxa and their surrounding environment. In the following sections, I reflect on the results of the restoration efforts as well as the monitoring methods employed, discuss implications for the management of geogenic reefs in the Baltic Sea and briefly outline a number of potential future applications in which I foresee that geogenic reefs will become increasingly important.

7.1 Evaluation of restoration and monitoring methods

7.1.1. Restoration and management of geogenic habitats in the Baltic Sea
Following the successful large-scale restoration of a boulder reef in the Kattegat in 2008 (Støttrup et al., 2017), this thesis has evaluated the restoration of a boulder reef on a smaller scale (~ 2,000 m^3 of boulder used compared with 58,896 m^3 for the Kattegat reef) carried out in the WBS in early 2017. The volume of boulders was divided among four restoration sites (i.e. 500 m^3 of boulders per site), with reefs at two sites restored in a Single Large (SL) design and at the remaining two sites in a Several Small (SS) design to test whether Single Large or Several Small (SLOSS) reefs most benefited the associated marine community. In general, the restoration showed strong positive effects on predatory fishes, with a 60-fold and 129-fold increase in the relative abundance of gadoids (*Gadus morhua*, *Pollachius virens* and *Pollachius pollachius*) at SL and SS respectively, in less than six months after the reefs were restored. This result is promising in light of increasing calls for adaptive management strategies to recover predatory fish stocks and thereby reinstate the trophic integrity of coastal systems (e.g. Eriksson et al., 2011; Östman et al., 2016; Norderhaug et al., 2021). Restoring boulder habitats for predatory fishes could thus be an important facet within a holistic ecosystem-based approach to recover the resilience of coastal habitats (Curtin & Prellezo, 2010). However, it should be emphasized that our results are reflective of a system that is limited in hard-bottom habitat and was subjected to historic removal and degradation of this habitat type. The construction of new reefs in regions with abundant structural complexity and high substrate availability may therefore not be viable approach, as such systems are unlikely to be limited in carrying capacity (Folpp et al., 2020) and thus represent the least likely scenario for productivity enhancement through habitat provision (Layman & Allgeier, 2020). This is an important notion, as a

failure to recognize appropriate locations for reef deployment is likely to compromise restoration outcomes (Komyakova et al., 2019) and could even induce high mortality rates among individuals attracted away from nearby natural reefs if the new habitat does not offer adequate structural complexity to function as refuge sites (i.e. ecological traps; Komyakova et al., 2021). Given the short period between restoration efforts and post-restoration monitoring (less than six months), it is evident that the large numbers of gadoids present at the reefs must have been attracted from nearby habitats instead of locally produced. Still, we selected our restoration sites based on evidence of historic boulder removal from archived nautical maps (Kruse, 2016), and utilized natural substrate (i.e. quarry rock) to mimic the composition and structural complexity of natural reefs. This implies there is substantial scope for enhanced productivity at the restored reefs over time (Layman & Allgeier, 2020) and that the reefs offer refuge through interstices, ledges and cavernous structures created by layering or stacking of the natural boulders (Takada, 1999; Støttrup et al., 2017). This stands in contrast to artificial reef designs e.g. Reef Balls that are commonly deployed with the aim to enhance fish productivity, yet these types of structures offer little structurally complexity (Hylkema et al., 2020) and may act as ecological traps causing high mortality rates for certain species (Komyakova et al., 2021).

Apart from the strong positive effects on gadoids, the restoration also positively affected a number of small-bodied taxa including wrasses, demersal gobies and sand gobies. While this result was expected for reef-associated wrasses and demersal gobies (comprised of *Gobiusculus flavescens* and *Aphia minuta*), it was most notable that effects were also positive for sand gobies (*Gobius niger* and *Pomatoschistus* spp.) despite the reefs covering substantial areas of soft-bottom habitat. Soft-bottom taxa are mostly ignored in studies evaluating artificial reefs, since these reefs are often deployed with the specific target of enhancing commercially important reef-associated taxa (e.g. Scott et al., 2015; Froehlich et al., 2019). However, a recent study by Puckeridge et al. (2021) revealed that a soft-bottom predator (bluespotted flathead *Platycephalus caeruleopunctatus*) was successfully using artificial reefs in Australian waters as forage grounds to predate on reef-associated zooplanktivores. This finding hints at the potential existence of bentho-pelagic trophic links facilitated by the reef structures, which if present would imply that the productivity of artificial reefs may currently be largely underestimated due to exclusion of soft-bottom predators in the majority of monitoring studies (Puckeridge et al., 2021). The positive effects on soft-bottom taxa in our study was limited to small-bodied sand gobies (only observed at SS reefs), while large-bodied flatfishes were observed in lower abundance at both reef types relative to pre-restoration sites. We acknowledge however, that underwater video monitoring may not be the optimal method to survey ambush predators relying on cryptic behavior and generally exhibiting low mobility patterns. The use of acoustic tags by Puckeridge et al. (2021) revealed that flatheads were largely immobile during daylight hours and relying on visual ambush predation near the reefs while they were more active and further away from the reefs at night. If flatfish in our study were similarly exhibiting low mobility near the reefs during daylight hours, there is a chance that we substantially underestimated

flatfish abundance by focusing on diurnal patterns, which should be investigated in follow-up studies on the restored reefs using e.g. acoustic telemetry or RUVS with artificial light sources deployed at night.

Overall, the restoration of SS reefs resulted in a higher abundance of several focal taxa relative to SL, while the remaining taxa were equally abundant at both reef designs. However, since all the taxa promoted at SS reefs were small-bodied mesopredators, future development of these reefs will likely strongly depend on dynamics of top predators (e.g. cod) and their ability to sustain trophic integrity through top-down control on mesopredators. This thesis work therefore demonstrates that the SLOSS concept (and spatial habitat configuration in general) could have important implications for the long-term resilience of restored habitats, which will be further investigated in subsequent monitoring studies.

Our effort to restore cobble reefs in the WBS represents, to the best of our knowledge, the first attempt to restore this habitat type in temperate marine waters. While the restoration of cobble reefs did not result in a 'textbook' increase in taxonomic diversity and species abundances relative to pre-restoration conditions, our BACI sampling design revealed that the restoration did partly alleviate a general decreasing trend in these response variables across the bay. In particular, the diversity of marine fauna (expressed in Hill diversity metrics) decreased between the two sampling years, which was most notable at natural reefs, yet the restored cobble reefs counteracted similar decreases at restoration sites. The restored cobble reefs hosted a unique species composition (driven mainly by small-bodied benthic and demersal gobies) that diverged away from the pre-restoration assemblage characterized by soft-bottom taxa, and converged slightly towards the natural reef assemblage (although still clearly being distinct). The use of correlated response models (CRMs) further indicated that variations in cobble coverage on the seabed explained a large number of positive and negative correlations among taxa in our study area, highlighting the importance of this habitat type for a wide variety of organisms. While we did not find any effect of reef height on the early stages of colonization following the restoration efforts, the long-term effects of height within cobble reef systems remains unknown and should be determined in follow-up studies, given the important role of light intensity in shaping macroalgal communities (Markager & Sand-Jensen, 1992).

Natural cobble reefs within the Baltic Sea originate mainly from glacial till deposits, as previously discussed in Chapter 1. However, due to the smaller size of individual rocks relative to boulders (Table 1.1), cobble substrate constitutes a highly dynamic type of habitat with physical characteristics and colonization rates being governed by various wave-induced processes (von Rönn et al., 2021). Individual cobbles within coastal areas may experience high rates of overturning by wave forces depending on the diameter of the rocks, water depth and wave energy input (Davis & Wilce, 1987; Schrottke et al., 2006). In addition, algal-mediated displacement of cobbles may occur after attached macroalgae reach a critical mass, resulting in overturning and displacement of cobbles by drag forces (Scheibling et al., 2009). While frequent overturning interrupts the successional sequences of colonizing algae and sessile animals, there is evidence that intermediate overturning may result in more diverse assemblages, relative to seldom or

frequently overturned rocks generally characterized by a few dominant species (Sousa, 1979). Combined, these types of physical disturbances highlight a particular challenge associated with restoring cobble habitats relative to heavier and more stable boulder habitats, which is that of long-term structural integrity and reef stability. At the time of post-restoration sampling of the cobble reefs (i.e. 4 months after restoring the reefs), we found no evidence of displaced cobbles next to the restored reef units and therefore no signs of destabilizing reef slopes by overturning cobbles. There is a possibility that the design of the restored cobble reefs used in our study, with cobbles tightly packed in truncated pyramid reef units (Fig. 5.1), prevented overturning by reducing hydrodynamic forces exerted on the reef relative to individual cobbles on the seabed. However, the short period between restoration and sampling (4 months) also implied that the newly restored reefs were dominated by ephemeral algae mats, and potential succession of upright perennial macroalgae on the restored reefs could increase the risk of destabilization over time by rendering individual cobbles more susceptible to displacement (Scheibling et al., 2009). We therefore recommend similar future restoration efforts to employ long-term monitoring of restored cobble (or small boulder) reefs, to evaluate the succession of colonizing species and potential consequences for reef stability.

Given the historical excavations of hard substrates in the Baltic Sea (as outlined in Section 1.2.3) and the concurrent loss of reef habitat for coastal marine species over many decades, it is a somewhat promising notion that hard-bottom habitats are also being naturally regenerated in this region (Bohling et al., 2009; Schwarzer et al., 2014; von Rönn et al., 2021). In particular, ongoing natural abrasion at rates of 1-5 cm/yr^{-1} continues to expose glacial till in WBS coastal waters down to 7 m of water depth and have led to an increase in the amount of hard substrate in the region over the past three decades (Schwarzer et al., 2014). Episodic retreat of active cliffs along the German Baltic coast triggered by marine forces (e.g. storm surges) also exposes glacial till (Averes et al., 2021), although the exact contribution of this process to the availability of cobble and boulder substrates is still poorly understood and warrants more research. Upon exposure, wave-induced forces actively rework the glacial till and transport the sediment away from the source area depending on the grain size of the material. Interestingly, von Rönn et al. (2021) have shown that transportation of cobbles can create new habitat (i.e. 'autochthonous' habitats) or extent existing hard-bottom areas, in contrast to 'allochthonous' habitats formed close to the source (e.g. by heavier boulders). This opens up new opportunities and challenges for the conservation of geogenic habitats in the Baltic Sea. In general, the effective management of existing and newly established hard-bottom habitats will require detailed sediment distribution maps on which reef habitats are accurately demarcated, and which are actively updated given the dynamic nature of these habitats as described above. However, the detection and mapping of cobble and boulder habitats is currently based on manual identification using backscatter mosaics from acoustic surveys (e.g. sidescan sonars or multibeam echo sounders), which is a time-demanding and costly process (Papenmeier & Hass, 2018). Ongoing research into the development of automated detection methods (e.g. using Haar-like features or neural networks; Feldens et al., 2019; Michaelis et al., 2019) therefore has much to offer in terms of accurate detection and

mapping of cobble reefs, and may ultimately prove paramount in the conservation of these dynamic habitats. Despite the banning of boulder extraction from Danish waters in 2010 (Kristensen et al., 2017), the extraction of cobbles and finer sediments remains legal and continues in dedicated coastal areas (e.g. during mussel dredging; Nielsen et al., 2020). While this thesis has demonstrated that cobble and boulder reefs can be successfully restored to the benefit of coastal marine communities, continued conservation and restoration efforts are thus warranted, which should be guided by frequently updated sediment maps and empirical evidence from field studies.

7.1.2. Evaluation of non-invasive monitoring techniques

Efforts aiming to preserve or restore marine ecosystems demand accurate knowledge on the occurrence, abundance, behavior and distribution of marine organisms and how these attributes are affected by management interventions. Ideally, the techniques employed to meet this purpose should offer a non-invasive assessment of marine organisms (i.e. organisms remain undisturbed during sampling) and provide a rapid, cost-effective assessment of target and reference sites over a prolonged period. In this thesis, a number of non-invasive monitoring methods were used to sample the marine community associated with restored reefs and reference sites within a Natura 2000 protected coastal area. In particular, I have focused on remote underwater video systems (RUVS), a method early on considered as an alternative to long-line (Ellis & DeMartini, 1995) and demersal trawls (Priede & Merrett, 1996) that are unsuitable for monitoring protected areas (e.g. MPAs) or restored habitats due to their extractive nature. Chapter 4 demonstrated that the addition of bait to a RUVS setup (i.e. BRUVS) did not obscure species-habitat associations (SHAs) for a number of prominent coastal marine species in the WBS. This finding is relevant in particular to address previous concern regarding the potential bias in SHAs as documented by BRUVS, possibly induced by attracting species away from their preferred habitats and into the camera's field of view (Hannah & Blume, 2012). Apart from capturing similar SHAs for four out of seven focal species compared with unbaited cameras, BRUVS captured SHAs for two additional focal species (*Gadus morhua* and *Merlangius merlangus*) that were underrepresented (i.e. inflated zero counts) in the unbaited samples. The addition of bait also provided additional insights into species-specific SHAs, revealing unimodality in the relationship between seabed cobble coverage and cod abundance, which peaked at 65-70% seabed coverage indicative of reef mosaics surrounded by soft bottom habitat. These findings highlight the potential to use BRUVS for documenting SHAs and corroborate the benefit of a bait attractant to reduce variation in MaxN counts and thereby increase the power of statistical inference relative to unbaited cameras (Cappo et al., 2003; Watson et al., 2005; Bernard & Götz, 2012). However, it also remains important to highlight a number of benefits related to the use of unbaited systems that emerged from this thesis work.

Unbaited cameras used for monitoring in Chapters 3 and 4 were equipped with a timer (Time Lapse Intervalometer, CamDo Solutions, Canada) that triggered the camera to turn on, record and turn off again, and thereby enabled the recording of short hourly video sequences (i.e. two-minute recordings

per hour). The use of timed interval (or time-lapse) recordings is relatively uncommon in underwater video monitoring, mainly due to the majority of studies employing BRUVS which requires continuous recordings to capture the bait plume effect before complete dilution. Still, a few other studies have previously adopted this method to allow for delayed, synchronized recordings while extending battery life across the diel cycle (Myers et al., 2016; Paxton et al., 2018; Mercaldo-Allen et al., 2021). For example, Mercaldo-Allen et al. (2021) utilized the technique to delay initial recording for 24h and minimize deployment-related disturbances within oyster cages, while Myers et al. (2016) timed their recordings to synchronize with a blue LED light to allow for nocturnal sampling of temperate reef fishes. While our deployment units did not include artificial light sources, implying that night recordings had to be discarded, the timers did allow us to monitor diurnal and crepuscular patterns at the study areas without the need for continued presence of field staff to retrieve and re-deploy the camera units (as would be the case when using BRUVS). In addition, with batteries and SD card capacities allowing for coverage of up to 64 hours (and up to 120 hours in follow-up monitoring using new cameras and timer models; not included in the present thesis), this technique also allows for continued monitoring in severe weather conditions during which field expeditions would not be feasible. There generally remains a potential bias in marine monitoring studies towards sampling in good weather conditions instead of a priori randomization of sampling periods (this also applies to e.g. dive surveys or surveys conducted from small vessels; Willis et al., 2000; Poulard & Trenkel, 2007), which could be investigated in future studies by using remote sampling techniques, e.g. time-lapse videos.

While the timers allowed for the collection of 24-60 samples per camera deployment and thereby resulted in thousands of individual samples during monitoring of the restored boulder reefs (Table 3.1), this thesis also identified a number of statistical challenges arising from the use of hourly unbaited video sequences. Due to the relatively short duration of individual hourly sequences (here two minutes), many of the samples can be expected to result in zero counts for the response variable. This is a challenge that is generally associated with unbaited video methods (Cappo et al., 2003), yet statistical approaches have been developed in the past decade to capture excessive zero counts in a flexible way (Garay et al., 2011; Zuur & Ieno, 2016). For example, generalized linear models (GLMs) for count data (e.g. using a Poisson or negative binomial distribution) can be tested in their capacity to capture zero counts via direct simulations or model diagnostics (e.g. Fig. S3.5) and be replaced by mixture models (e.g. zero-inflated Poisson or zero-inflated negative binomial models) if diagnostics indicate this capacity is inadequate. Furthermore, while GLMs are based on the underlying assumption that model residuals are independent and identically distributed (Wikle et al., 2019), this will often not be the case for hourly recordings produced by stationary video systems. For example, the probability that a highly resident reef fish (e.g. goldsinny wrasse) is recorded on a particular video sequence is likely to be higher if the video sample of the previous hour also recorded the resident fish and vice versa. The statistical models employed in Chapter 3 explicitly deal with this temporal dependence among residuals if present, as a first-order autoregressive structure (i.e. y_t depends on y_{t-1}) is included those cases, implying counts from an hourly

video sequence are regressed against regular predictors as well as counts from the previous sequence. Finally, the use of hourly time-lapse videos will often induce inter-site variabilities in sampling effort by recording differential numbers of hourly sequences across deployments due to variations in battery life (here this resulted in variations between 24 and 60 recordings per camera). In Chapter 3, I have demonstrated how to deal with resulting unequal sample sizes by using hierarchical models and defining grouping-level effects for each camera deployment to be nested within the respective field sites. In this way, unique intercepts are estimated for each camera deployment within the lowest model hierarchy and information is shared among deployments from the same field site (i.e. partial pooling) to estimate a population of varying intercepts (McElreath, 2016; Nalborczyk et al., 2019). This is one of the most essential features of multi-level modelling and yields more robust parameter estimations given repeated measurements or unbalanced designs relative to single-level regression models (Nalborczyk et al., 2019). Collectively, Chapters 3 and 4 therefore demonstrate that the use of repeated time-lapse recordings can overcome the challenges of low statistical power and inflated zero counts (by sampling frequently over prolonged periods) that are typically associated with continuous unbaited video monitoring, given that the dependence between hourly recordings is adequately accounted for through appropriate model structures. Finally, one important limitation of the video methods used in the present thesis was the mono-camera setup. The use of stereo-video systems, whether baited or unbaited, opens up a number of interesting avenues in underwater video monitoring research that may allow for more informative inference from video data. In particular, the ability to accurately measure body size from calibrated stereo images (Harvey et al., 2001; Langlois et al., 2020) facilitates the delineation of life stages and enables a conversion to fish biomass via established length-weight relationships for fish species (available on e.g. FishBase; Froese & Pauly, 2020). Body size correlates with many important aspects related to e.g. growth, feeding and mortality rates, and is accordingly often considered a 'master trait' (Ohman & Romagnan, 2016; Kiørboe et al., 2018). Accurate size measurements thereby allow for a characterization of fish communities based on traits and comparisons of functional diversity metrics between different systems (Ford & Roberts, 2020; Myers et al., 2021). Biomass can be used to estimate fish productivity using growth trajectories and mortality rates predicted from shape and life history traits, which can be aggregated across multiple spatial or temporal levels to estimate the productivity of reef systems (Morais & Bellwood, 2020). These avenues merit wider incorporation into future RUVS studies and could contribute significantly to our current understanding of the functioning and productivity of coastal marine habitats.

Environmental DNA (eDNA) sampling conducted in Chapter 6 revealed a number of advantages and limitations relative to video monitoring. A particularly noteworthy advantage of eDNA sampling relative to BRUVS was the significantly higher sensitivity (i.e. species detection) offered by the eDNA method, including for cod which are easily detected using BRUVS due to their highly conspicuous appearance and generally strong affinity for the bait. In addition, we provide evidence of highly localized eDNA traces for species exhibiting high residency (goldsinny wrasse; Halvorsen et al., 2021) or a

sedentary lifestyle (flounder; Gibson, Stoner, & Clifford, 2005), as eDNA sampling successfully identified habitat associations for these species in our study area characterized by a mosaic of coastal habitats. Although likely depending on mobility and lifestyle of the target species, this result highlights that eDNA sampling can detect SHAs on small spatial scales despite the dynamic nature of the marine environment, which is in agreement with results from similar recent comparative studies (Port et al., 2016; Stat et al., 2019; Oka et al., 2021). One limitation of the eDNA method we encountered in our study was the inability to treat the data quantitatively, due to detected DNA concentrations being below the limit of quantification (LOQ), implying a lack of precision that necessitates qualitative data treatment (Klymus et al., 2020). We note that this limitation most likely arises from inherent low species concentrations in the study area of the WBS relative to less degraded areas, yet adjustments to the sampling regime and laboratory methods could potentially alleviate the issue of low DNA concentrations in the samples. The yield of target DNA likely correlates positively with the volume of water that is sampled (Hunter et al., 2019) and with the number of PCR replicates performed in the laboratory (Ficetola et al., 2015). We therefore recommend that future studies from similar systems use volumes > 1 L (e.g. 3-5 L) and a higher number of replicates (e.g. 6-12 replicates), to reduce the risk of target DNA concentrations falling below the LOQ and precluding a quantitative assessment. Sampling volumes in aquatic studies typically range from 15 mL to 6 L due to clogging filters at higher volumes caused by sediment or biological particles (e.g. algae; Hunter et al., 2019). However, sampling and filtration methods are constantly improved (e.g. a recently developed mesh tow method allows for samples of > 3000 L in lentic systems; Schabacker et al., 2020) and ongoing developments will likely enable low-cost sampling of larger water volumes in the near future. We subsequently explored a 'semi-quantitative' method by expressing eDNA signals as proportions of qPCR replicates that amplified (i.e. indicating a positive detection of target DNA within the replicate) rather than mere presence-absence signals (producing either a 0 for non-detections or a 1 if one or more replicates amplify). Using zero-one inflated beta models, we detected effects of temperature on absent (0 out of 3 replicates) and full (3 out of 3 replicates) amplification for two focal species, yet similar SHAs were detected compared with the presence-absence method for all species. However, we argue that SHAs will likely be obscured in presence-absence data when studies increase the number of PCR replicates to improve species detection, as it will be more likely that one or more replicates amplify when using e.g. twelve replicates relative to the three replicates used in our study (Furlan et al., 2016). Our semi-quantitative method could therefore prove beneficial for studies using a high number of replicates (but with concentrations below the LOQ), by providing a higher data resolution to detect differences in eDNA signals relative to presence-absence data, which should be investigated in future studies. Finally, we hope that the species-specific assay for goldsinny wrasse (*Ctenolabrus rupestris*) developed in Chapter 6 will facilitate improved future monitoring of this temperate reef species. The goldsinny wrasse is increasingly exploited to serve as a cleaner fish in the salmonid aquaculture industry (Blanco Gonzalez & de Boer, 2017; Moland et al., 2021), which is already having an impact on the abundance of goldsinny wrasse along the southern Norwegian coast (Halvorsen et al., 2017). Ultimately, eDNA research has

much to offer both in terms of species-specific monitoring and community-wide assessments to better understand marine biodiversity levels and inform conservation efforts (Lotze, 2021).

7.1.3. Flexibility and intuitive inference offered by MCMC sampling and BACI ratio computation
Bayesian statistics are becoming increasingly popular among scientists working in applied fields (e.g. psychology or biology) and benefits relative to frequentist approaches have been widely discussed, including the ability to quantify evidence in support of a null hypothesis, the potential to incorporate prior knowledge of the study system into the analysis and the high degree of modelling flexibility obtained from relying on established probability rules (Wade, 2000; Clark, 2005; Golding & Purse, 2016; Keysers, Gazzola, & Wagenmakers, 2020). A crucial difference between the two approaches is the way in which probability is defined and estimated. While frequentist approaches involve estimating the probability of observing the data given a particular hypothesis, or $P(Y \mid \mathrm{H})$, Bayesian inference estimates the probability of a hypothesis of interest given the data that has been collected, or $P(\mathrm{H} \mid Y)$ (Ellison, 2004). In the context of impact assessments conducted in environmental studies (e.g. using a BACI design), the Bayesian approach therefore offers an intuitive interpretation of results by enabling researchers to report on the probability that effect sizes of interest are observed in the field (Crome, Thomas, & Moore, 1996). Using habitat restoration central to this thesis work as an example, the primary research question of interest often involves the likelihood of restoration efforts to positively affect the response variable of interest (e.g. biodiversity or the abundance of target species). This likelihood, or the probability of a positive restoration effect, can be computed directly using Bayesian methods whereas the interpretation of traditional p-values (i.e. the probability of observing the data, or more extreme data, given the null hypothesis of 'no effect') can be inherently challenging to convey to e.g. restoration managers, stakeholders or decision makers (Crome et al., 1996; Wade, 2000; Conner et al., 2016). In Bayesian models, Markov Chain Monte-Carlo (MCMC) sampling is used to estimate model parameters by exploring the parameter space through random sampling (Monte-Carlo) based on a sequential process that defines a stepping stone from one random sample to the next (thereby forming the Markov Chain; Fig. 7.1; van Ravenzwaaij, Cassey, & Brown, 2018). Throughout this thesis, we have utilized these individual MCMC samples to compute BACI ratios via the equations presented in Chapter 3 (based on the method described in Conner et al., 2016), which represent proportional response variables that reflect the difference between control and impact sites across time. For example, a BACI ratio of 1 implies that this difference remained unchanged after restoring the reefs, whereas a ratio of e.g. 1.50 indicates a 50% increase in the response variable following the restoration efforts. By plotting the entire distribution of the BACI ratios, consisting of *N* ratios depending on the number of post-warmup iterations (Fig. 7.1), the density of ratios corresponding to a specific hypothesis (e.g. Table 6.2) can be clearly visualized and expressed in probabilities by computing the area under the curve. Such an approach can also be useful outside of restoration ecology, e.g. to examine the probability that monthly culling efforts are achieving the
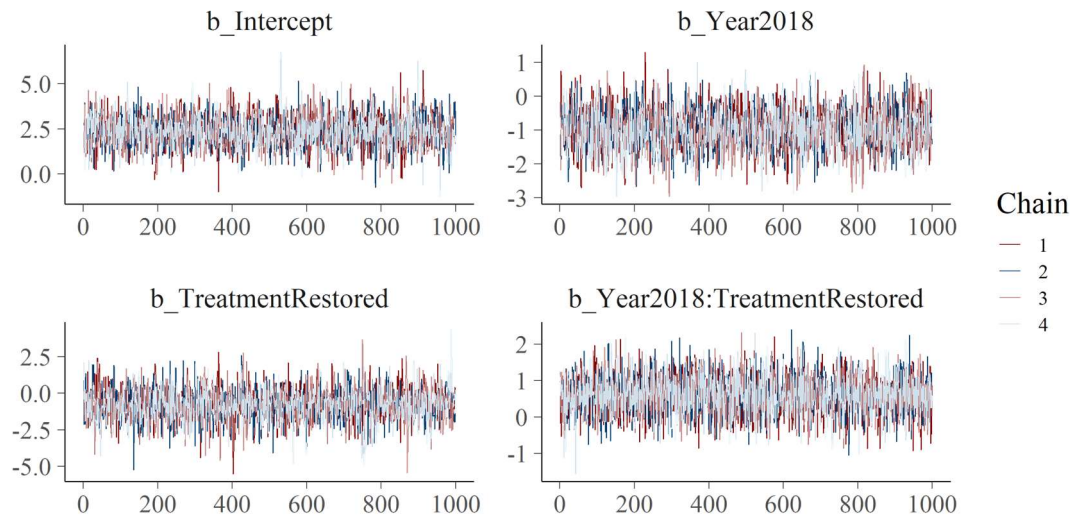
Figure 7.1 – Example of trace plots visualizing MCMC chains for the BACI parameters in a Bayesian model. Parameter estimates are based on random sampling of the parameter space (with parameter values denoted on the y-axis, here on the log-scale) based on a sequential process that forms the Markov chains. Note that only 1000 post-warmup iterations (x-axis) per chain are used in this example to optimize visualization of the chains, yet we generally ran at least 10,000 post-warmup iterations per chain in the models used throughout this thesis to ensure convergence and reliable parameter estimation. The individual MCMC samples obtained per chain and iteration were used to compute posterior ratios reflecting the difference between treatments across time (i.e. BACI ratios; Chapter 3 & 5), or the effect of habitat type and sampling method on the detection of target species (Chapter 6).

desired reduction in density of an invasive species (Johnston & Purkis, 2015), given the monitoring data that is collected. The combination of MCMC sampling and BACI ratio computation therefore produces clear metrics that are highly suitable for decision making, by framing monitoring results as the probability that an effect size deemed important by e.g. managers or stakeholders has in fact occurred (Conner et al., 2016).

Finally, while restoration efforts in the current thesis work were only based on a single post-restoration monitoring period, the iterative nature of Bayesian statistics also allows for updating of our knowledge on the study system in multi-year monitoring studies (i.e. 'Bayesian updating'). This is achieved by sequentially using the posterior distributions of model parameters from previous monitoring studies as data-based prior distributions in subsequent studies (Wade, 2000; Ellison, 2004). Ultimately, Bayesian updating thereby facilitates adaptive management strategies as system knowledge is accumulated over time, while the consequences of future management actions can be predicted based on the current level of knowledge and estimated uncertainties (Dorazio & Johnson, 2003; McCarthy & Possingham, 2007). As statistical software supporting Bayesian methods are becoming widespread and computational efficiency is continuously improved (Albers, Kiers, & Ravenzwaaij, 2018), Bayesian

statistics will likely be increasingly embraced by environmentalists and we argue that the method has much to offer both in terms of modelling flexibility and intuitive probabilistic inference.

7.2. Future perspectives

Given the urgent ecological and socio-economic challenges that humanity faces in the 21st century, it is now more critical than ever to prioritize sustainable resource use and to promote innovative research aiming to identify and realize the benefits people receive from nature (i.e. ecosystem services). To this end, the European Commission is investing heavily in research and innovation through the Horizon 2020 initiative, which facilitates large-scale pilot studies testing tangible nature-based solutions to pressing societal challenges (European Commision, 2015; Maes & Jacobs, 2017). I envision that geogenic reefs will become increasingly important in the field of ecological engineering (i.e. designing ecosystems for the mutual benefit of humans and nature; Bergen, Bolton, & Fridley, 2001), by providing essential habitat for the conservation of marine species and biodiversity, while offering benefits to society via coastal protection and other important ecosystem services. Rising sea levels and an increasing frequency of storm surges are destabilizing coastlines around the globe and jeopardizing coastal communities and their properties (Vellinga & Leatherman, 1989; Dasgupta et al., 2011). There is now a growing interest in the conservation and restoration of coastal habitats (e.g. seagrass meadows, mangroves and reefs) for their service of providing coastal protection by attenuating wave energy and thereby maintaining shoreline integrity and resilience (Sutton-Grier et al., 2015; Narayan et al., 2016). In the U.S. for example, coastal habitats currently protect 67% of the total coastline and the loss of these protective habitats would double the amount of people highly exposed to coastal hazards (Arkema et al., 2013). In Denmark, the erosion of coastlines is a growing problem that is currently addressed by dredging millions m^3 of sand from offshore areas and depositing the sand directly on an eroding shore (i.e. beach nourishment; Speybroeck et al., 2006). Apart from widely documented negative impacts on resident flora and fauna (e.g. suffocation under nourishment sand; Speybroeck et al., 2006; Schlacher et al., 2012), increasing storms and rising sea levels imply that beach nourishment efforts would need to intensify accordingly to maintain coastal stability. Currently, beach nourishment is often repeated every 3-5 years already at individual coastal areas, with associated costs approaching 1 million kroner per year per kilometer of beach (Kristiansen, 2016) and further intensification is therefore unlikely to be sustainable. The construction of geogenic reefs along Danish coasts has the potential to offer a sustainable supplement or even an alternative to beach nourishment (Fig. 7.2). Vertically protruding reef structures could naturally attenuate wave energy and protect the shoreline from high water velocities, while increasing the structural complexity of the seabed and thereby enhancing food and shelter availability for coastal species. The presence of well-developed reef systems has previously been linked to preservation of natural coastlines and biodiversity in tropical islands on the front lines of climate change, relative to nearby coastal bays with degraded reefs that

experienced severe erosion (Reguero et al., 2018). Depending on local hydrodynamic conditions, the hard substrate provided by geogenic reefs could concurrently promote settlement and development of biogenic elements (e.g. mussel reefs or macroalgal beds), while the sheltered low-energy environment behind the reef provides favorable conditions for the development of eelgrass meadows by reducing seedling uprooting by wave forces (Kuusemäe et al., 2016). Biogenic reef elements provide beneficial regulatory services, e.g. water filtering and carbon sequestration (Krause-Jensen & Duarte, 2016; zu Ermgassen et al., 2020), and provisional services in the form of food supply and nursery habitats (Gilby et al., 2018; Du et al., 2020; Fulton et al., 2020). Accordingly, the construction of geogenic reefs along eroding coastlines offers a promising alternative to unsustainable beach nourishment practices or artificial structures that offer little structural complexity, and this concept will therefore be tested through a real-world implementation along the Danish coastline in the near future.
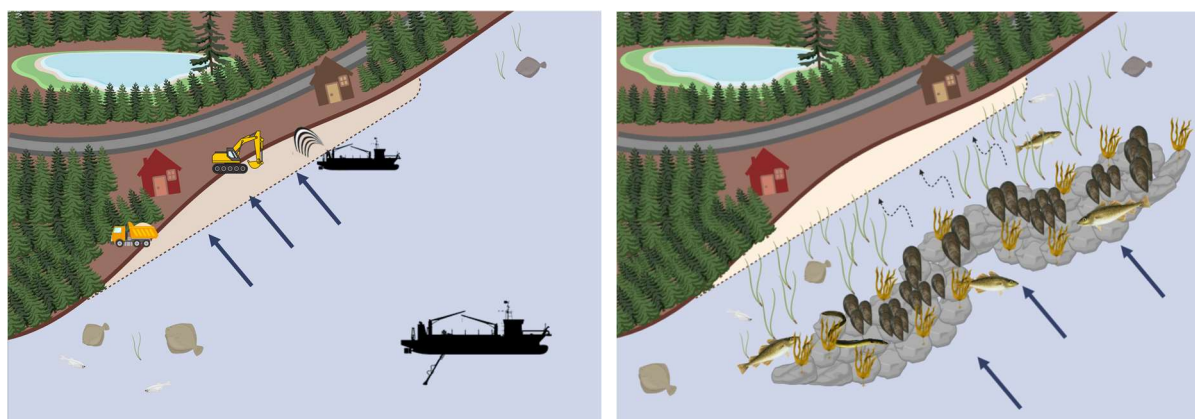

Figure 7.2 – Conceptual illustration of the current solution to erosion along the Danish coastline (left) and a proposed nature-based solution through coastal reef construction (right). The protruding vertical reef structures induce natural attenuation of wave energy, while the presence of a well-developed coastal reef system promotes marine biodiversity and offers beneficial ecosystem services to society (see main text). Created with BioRender (www.biorender.com).

Geogenic substrates will also become increasingly important for the diversification of infrastructure in the marine environment. In response to ongoing climate and ecological crises, there is current momentum to extend existing governmental policies of 'No Net Loss' (NNL) from infrastructure projects in the marine environment, towards one of 'marine net gain' (Hooper, Austen, & Lannin, 2021). The NNL concept implies that environmental impacts from developments should first and foremost be avoided, otherwise be minimized if unavoidable with residual impacts being offset (i.e. the mitigation hierarchy; Arlidge et al., 2018). The concept of net gain would extent this hierarchy, by requiring a final step of ensuring that the development results in wider environmental benefits (e.g. increasing ecosystem services, removing threats; restoring natural capital assets; Hooper et al., 2021). For example, offshore wind farms can act as artificial reef systems by providing hard structures to the benefit of reef-associated communities (Langhamer, 2012). Although such artificial structures do not mimic reef systems that were

historically present (e.g. oyster reefs in the North Sea; Fariñas-Franco et al., 2018), they have the potential to offer some of the lost ecological functions and services (e.g. habitat and nursery provision; Hooper, Beaumont, & Hattam, 2017). Importantly, the progression towards marine net gain will require a diversification of such structures, including e.g. groynes and seawalls (Chapman & Underwood, 2011), breakwaters (Mamo et al., 2021) or scour protection surrounding wind farms (Glarou, Zrust, & Svendsen, 2020), to further enhance their structural complexity and thereby the potential to provide suitable habitat for a wider range of biota, ultimately to the benefit of marine biodiversity. Diversifying artificial structures will likely be increasingly achieved using geogenic substrates of various sizes, because these substrates offer multiple benefits in the form of longevity, mimicking natural habitats and the ease with which they are further manipulated, e.g. by drilling holes to create diverse rock pools (Firth et al., 2014; Evans et al., 2016) or by tailoring shelter sizes to fit target species (Glarou et al., 2020). The provision of additional ecosystem services, e.g. water filtering and carbon sequestration by biogenic reef elements as discussed above, will ultimately further contribute towards the goal of marine net gain. However, it should be emphasized that the concept of marine net gain is very much in its infancy and there are many substantial barriers to overcome before potential widespread implementation can be achieved. Despite of the early emergence of the NNL precursor in the late 80s (Jacob et al., 2018), there are remarkably little examples of successful implementations for the marine environment (Bull & Strange, 2018) with a clear lack in guidelines on how to pursue mitigation (Jacob et al., 2018) and measure offsets (Jacob, Pioch, & Thorin, 2016). Current monitoring standards are also below the required standard, with monitoring typically conducted for less than one year during the construction phase of projects and not extended into the operational phase (Jacob et al., 2018), while coastal ecosystems are expected to require 15-25 years to recover from degradation (Borja et al., 2010). It may therefore be premature to suggest that offsetting can even be effective in marine environments (Shumway et al., 2018) and the net gain concept is therefore set to inherit many of these unresolved challenges. Indeed, at present, the formal definition of net gain remains largely unclear and there are no transparent guidelines on how it should be measured in marine environments (Hooper et al., 2021). Collectively, the challenging task of moving forward toward marine net gain will therefore not only require the formulation of clear goals, performance standards and suitable metrics, but also continued long-term monitoring efforts to evaluate how the various approaches actually contribute to healthier marine ecosystems in practice.

Given the potential to use geogenic substrates for multi-faceted purposes such as (co-)restoring marine habitats, maintaining shoreline integrity and enhancing existing artificial structures, it is evident that future empirical studies should continue to guide such efforts to test theories and concepts, identify successes and failures, and ultimately optimize the intended benefits to nature and society (Schoonees et al., 2019; Glarou et al., 2020; Vivier et al., 2021). Currently, the use of geogenic substrates (e.g. to serve as a foundation for new shellfish reefs) often remains unreported and is mainly done to cut costs without proper acknowledgement that multiple unique habitat types are being restored (Liversage, 2020). Such a focus on the 'target habitat' (in this case shellfish reefs) implies that potential side benefits provided by the

geogenic substrate are not recognized and conversely, existing knowledge on geogenic reef design may not be incorporated (Liversage, 2020). This could not only reduce the efficacy of current restoration practices, but also hamper the development of future efforts through limited interdisciplinary knowledge exchange. At the very least, efforts incorporating geogenic substrates to restore habitat or diversify marine infrastructure should report on the structural design and type of material used, such that the implementation and potential effects are documented and can be synthesized (e.g. in meta-analyses; Vivier et al., 2021). Ultimately, this improves our ability to realize the wide range of benefits that geogenic reef systems have to offer and provides crucial information to guide sustainable management strategies in the face of accelerating global change.

7.3. Conclusions

In summary, this thesis has demonstrated that geogenic reef restoration is a promising tool to aid the recovery of commercially important cod and other gadoids in the Baltic Sea, and can alleviate local declines in species diversity in degraded coastal systems. Our results highlight the importance of hard-bottom substrates and structural complexity for a wide range of Baltic marine taxa and call for increased efforts to conserve and restore this important habitat type, including reef habitats consisting of small rocks (diameter < 26 cm) that currently lack protective status and are still extracted from Danish waters. The spatial configuration of restored boulder reefs was found to strongly affect the abundance of small-bodied mesopredators, with potential long-term implications for the resilience of restored marine habitats that warrant further investigation. Future marine restoration efforts should continue to test the applicability of concepts and theories originating from other fields, such as terrestrial restoration or landscape ecology, to advance the relatively young discipline of marine habitat restoration and to guide effective allocation of limited resources aimed at optimizing outcomes. We further show that baited video sampling can effectively record associations of coastal species with surrounding habitat despite the use of bait attractant, and we foresee that this non-invasive method will play an important role in monitoring marine restoration efforts and similarly vulnerable marine habitats (e.g. protected areas). Emerging eDNA techniques have much to offer in terms of rapid, low-cost assessments that provide high species detection rates, and in-tandem use with established non-invasive methods can therefore provide a more holistic view on spatiotemporal distributions of marine taxa in relation to coastal habitat. Finally, we have demonstrated throughout this thesis how the robust BACI sampling design can effectively be coupled with a Bayesian statistical framework to provide intuitive probabilistic results that are easily interpreted and conveyed to all parties involved in the restoration efforts.

While the findings of this thesis are based on monitoring of a degraded coastal area in the western Baltic Sea, characterized by a unique multi-stressor situation, our results are likely to be relevant

for other coastal areas where availability of hard substrate is limiting, or for systems that may face similar cumulative stressors in the near future. The knowledge obtained from this thesis work is also relevant for studies in the field of ecological engineering, seeking to diversify artificial structures in the ocean for the mutual benefit of humans and nature.

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Supplementary contributions

Appendix A – Student projects

Casabona Balcells, E. (2021). Effects of reef restoration in the abundance of Atlantic cod *Gadus morhua* and Atlantic herring *Clupea harengus*. BSc thesis. **T.J.G. Wilms (co-supervisor)**

Pirou, O. (2021). Comparisons of fish abundance and diversity in designs of restored boulder reefs with baited and unbaited remote underwater video systems. BSc final internship report. **T.J.G. Wilms (internship supervisor)**

Stoehr. S. (2021). The effect of biogenic reefs on fish abundance and diversity and implications for coastal restoration. MSc thesis. **T.J.G. Wilms (co-supervisor)**

Norðfoss, P.H. (2019). Restoration of marine boulder reefs: Addressing the Single Large or Several Small (SLOSS) debate in relation to marine habitat restoration. MSc thesis. **T.J.G. Wilms (co-supervisor)**

Guay, A. (2019). Identifying the optimal boulder coverage for prey species of the harbor porpoise *Phocoena phocoena* in the Western Baltic Sea. BSc final internship report. **T.J.G. Wilms (internship supervisor)**

Groth, P.M. (2019). Utilisation of neural networks to count and identify marine species from underwater video recordings. MSc thesis. **T.J.G. Wilms (co-supervisor)**

Appendix B – Stoehr et al.

The effect of biogenic reefs on fish abundance and diversity and implications for coastal restoration (*in prep.*)

Svenja Stoehr, **Tim J.G. Wilms**, Linda Latuta, Jort Rootlieb, Alexandra Zachariadou, Julie Larcher, Karen Timmermann & Jon C. Svendsen

ABSTRACT

Complex coastal habitats such as reefs formed by the blue mussel *Mytilus edulis* provide a number of ecosystem functions to marine fauna, thus functioning as ecosystem engineers. Due to anthropogenic impacts including fishing and eutrophication, mussel reef distribution is decreasing in the Baltic Sea, indicating the need for evidence-based management strategies. The aims of this study were twofold: First, the ecological status of the study area was evaluated to assess whether it meets current European requirements set by the Water Framework Directive (WFD). Second, Baited Remote Underwater Video Systems (BRUVS) were used to investigate the mobile fauna diversity and species assemblage at mussel reefs compared to sandy bottom habitats. This study shows that despite poor ecological status, the mobile fauna found in mussel reefs is extremely rich and unique, indicating their importance as diversity hotspots. Efforts to reduce eutrophication need to be increased and the capacity of natural mussel reefs to mitigate eutrophication needs to be further investigated. Further, coastal conservation measures should be reconsidered to offer better protection to currently existing mussel reefs. In addition, future efforts should focus on large-scale restoration of bivalve reefs to improve coastal habitats and their ecological status.

Keywords

Water Framework Directive ● Ecological Status Assessment ● BRUVS ● Bivalve Reef ● Ecosystem Services

Appendix C – Bromhall et al.

Quantifying the impact of the Danish Seine - a 'low environmental impact' fishing gear - on seabed macrofauna (*in prep.*)

Katrina Bromhall, Ole R. Eigaard, **Tim J.G. Wilms**, Thomas Noack, Ciaran McLaverty, Finbarr G. O'Neill & Grete E. Dinesen

ABSTRACT

The Danish 'anchor' seine is an energy efficient commercial fishing gear used worldwide to catch demersal fish. This type of gear is assumed to have a comparatively low seabed impact due to its light weight and shallow penetration depth. Unlike other mobile bottom-contacting fishing gears, relatively little is known about the physical effect of Danish seining on benthic macrofauna. By undertaking single and multiple experimental trawls, we quantify the effect of the Danish seine's gear components on benthic macrofauna estimate using before-after control-impact ratios estimated from Bayesian models. We found higher depletion of benthic macrofauna from the seine ropes than the relatively heavier ground gear, and that the highest depletion occurred after multiple hauls of the seine ropes. Nonetheless, depletion of whole community indicators (density, species density and biomass) after multiple passes of the rope were low:-10%, -3%, -13%, respectively. Individuals of the tube-building *Phoronis* sp. exhibited high depletion (90%) after multiple passes of the seine rope. The generally low level of benthic macrofaunal depletion provides evidence that the Danish seine has a low seabed impact. We suggest that the use and promotion of fishing gears that have a low seabed impact can help to fulfil the objectives of an Ecosystem Approach to Fisheries Management.

List of acronyms

BACI	Before-after control-impact
BRUVS	Baited remote underwater video system
CI	Credible Interval / Confidence Interval / Control-Impact
CRM	Correlated response model
Ct	Cycle Threshold
eDNA	Environmental DNA
ESS	Effective Sample Size
FOV	Field of view
GAM	Generalized Additive Model
GLMM	Generalized Linear Mixed Model
GLMLM	Generalized Linear Multi-level Model
LOD	Limit of Detection
LOO	Leave-one-out
LOQ	Limit of Quantification
LVM	Latent Variable Model
MCMC	Markov Chain Monte Carlo
NNL	No Net Loss
NTC	No Template Control
qPCR	Quantitative Polymerase Chain Reaction
RUVS	Remote underwater video system
SFT	Space-for-time substitution
SHAs	Species-habitat associations
SLOSS	Single Large of Several Small
SST	Sea Surface Temperature

WBS	Western Baltic Sea
ZOIB	Zero-one Inflated Beta