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J. Kjerulf Petersen (ed.), J.L. Berthelsen, E.J. Brown, P.S. Freitas, T.G. Hansen, D.W. Hughes, D. O'Farrel, J. Olsen, J.C. Svendsen, K. Timmermann and T. Wilms

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Colophon

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Preface

In collaboration with several research institutions, the Limfjorden Council has in the period 2015-2021 managed the project "Investigation of the potential nitrogen effect of rocky reefs and contribution to the re-establishment of rocky reefs in the Natura 2000 area Løgstør Bredning, Vejlerne and Bulbjerg". As part of the project, a rocky reef was established northwest of Livø in Løgstør Bredning. The main aim of the project was to investigate to what extent the establishment of rocky reefs in certain parts of Løgstør Bredning could contribute to oxygenating the bottom water and thus retain nitrogen bound in the sediment. The results of this part of the project are reported elsewhere.

In addition, the project had the independent aim of establishing a rock reef in a Natura 2000 area and mapping its impact on local biodiversity. In this part of the project, the focus was initially on investigating the sedentary animal and plant life. Investigations showed that the area of the new reef contained both existing dense deposits of course substratum (mainly pebble) and areas of sand. For the sessile fauna, it was demonstrated that despite large variations between the individual samplings, the species diversity on the surrounding seabed with rocky material was high and was also higher at the sampling after the establishment of the rock reef than on the new rock reef. On the other hand, the biomass of mobile hard bottom fauna was greater on the reef than on the surrounding bottom. No macroalgae (kelp) were found on the bottom surrounding the established reef, probably due to a lack of light, while macroalgae were found on the reef at water depths down to a maximum of 4 m, albeit in limited densities.

It was decided at the beginning of 2021 to expand the studies of the associated biodiversity to also include mobile fauna with a focus on fish, lobsters, and shore crabs. In late summer 2021, two different types of surveys were thus carried out using different methods: a) investigation of the occurrence of European lobster and shore crabs using traditional fish stock assessment methods based on the capture of lobsters in nets and the use of tag-recapture techniques as well as sampling by means of fishing gear; and b) mapping of the occurrence of fish and biodiversity using underwater cameras. The investigations are reported in separate and independent sections for which the respective authors are responsible.

This report has in a draft version been send for commenting to Limfjordsrådet and Danish Environmental Protection Agencies. Inclusion of comments or suggestions for improvement of the text is however entirely the responsibility of the authors of the respective sections of the report.

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1. Dansk sammenfatning

Jens Kjerulf Petersen, Pedro S. Freitas, Jon C. Svendsen

1.1 Forord

Limfjordsrådet har i samarbejde med en række vidensinstitutioner i perioden 2015-2021 gennemført projektet ”Undersøgelse af stenrevs potentielle kvælstofeffekt samt bidrag til genetablering af stenrev i Natura 2000-området Løgstør Bredning, Vejlerne og Bulbjerg”. Som en del af projektet blev der efter en række forundersøgelser i 2017 etableret et stenrev nordvest for Livø i Løgstør Bredning. Projektet havde som hovedformål at undersøge, i hvilket omfang etablering af stenrev i bestemte dele af Løgstør Bredning kunne medvirke til at ilte bundvandet og dermed fastholde kvælstof bundet i sedimentet. Resultaterne af denne del af projektet er afrapporteret i Stæhr m.fl. (2020).

Derudover havde projektet som selvstændigt formål at etablere et stenrev i et Natura 2000-område og kortlægge dets betydning for den lokale biodiversitet. I denne del af projektet var der i første omgang fokus på at undersøge det fastsiddende dyre- og planteliv. Undersøgelser viste, at området for det nye rev både rummede eksisterende tætte forekomster af småstenede rev og områder udelukkende med sand. For den fastsiddende fauna blev det påvist, at trods store variationer mellem de enkelte prøvetagninger, så var artsdiversiteten på den omkringliggende havbund med stenet materiale høj og var ved prøvetagningen efter etablering af stenrevet også højere end på det nye stenrev. Til gengæld var biomassen af hårdbundsfauna større på revet end på den omkringliggende bund (Dahl m.fl. 2020). Der blev ikke fundet makroalger (tang) på den omkringliggende bund, sandsynligvis på grund af manglende lys, mens der på revet blev fundet makroalger på vanddybder ned til maksimalt 4 m om end i begrænsede tætheder (Dahl m.fl. 2020).

Det blev i starten af 2021 besluttet at udvide undersøgelserne af den associerede biodiversitet til også at omfatte mobil fauna med fokus på hummer, strandkrabber og fisk. I sensommeren 2021 blev der således gennemført to forskellige typer undersøgelser med brug af forskellige metoder: a) Undersøgelse af forekomst af europæisk hummer og strandkrabber med brug af traditionelle metoder fra fiskeriforskningen baseret på fangst af hummer i tejner og brug af mærkning-genfangst teknikker samt prøvetagning ved hjælp af fangstredskaber; og b) kortlægge forekomst af fisk og biodiversitet ved hjælp af undervandskameraer. Undersøgelserne er afrapporteret på engelsk i separate og uafhængige afsnit af denne rapport og sammenfattet på dansk nedenfor.

1.2 Indledning

Der er stigende interesse for at udlægge stenrev i danske farvande. Undersøgelser af stenrev ved Læsø og Sønderborg har illustreret, at stenrev kan være gavnlige for en række fiskearter som torsk, to-plettet kutling og havkarusse. Undersøgelser af udlagte stenrev har desuden dokumenteret højere forekomster af den lille hval marsvinet, og stenrev kan ligeledes forventes at give levesteder til større mobile krebsdyr som f.eks. hummere. Der er ønsker om at udlægge stenrev i mange danske fjorde, men effekterne af stenrev på den associerede mobile fauna i indre farvande som fjorde er dårligt belyst. Det nyetablerede stenrev i Løgstør Bredning udgør således et vigtigt bidrag til vores viden om effekter af habitatrestaurering.

Stenrevet i Løgstør Bredning fungerer i dag som et beskyttet område som funktion af et midlertidigt fiskeriforbud i området. Stenrevet kan dermed antages at have en positiv indflydelse på især bestanden af europæiske hummer ved at tilvejebringe levesteder samt beskyttelse mod fiskeridødelighed, men fiskeriforbuddet kan også antages at påvirke forekomst af fisk. Fremme af hummerbestanden i Limfjorden og skabelse af levesteder for fisk er helt i overensstemmelse med et af de oprindelige delformål med at etablere et stenrev. Effekten af beskyttede områder i fjorde som Limfjorden er imidlertid ukendt og revets interaktioner med tilstødende ikke-beskyttede områder og dets bestand er ligeledes ukendt. Da fiskeriforbuddet er midlertidigt og en evt. forlængelse eller et permanent forbud er betinget af mere viden, vil det være af største relevans at belyse disse forhold.

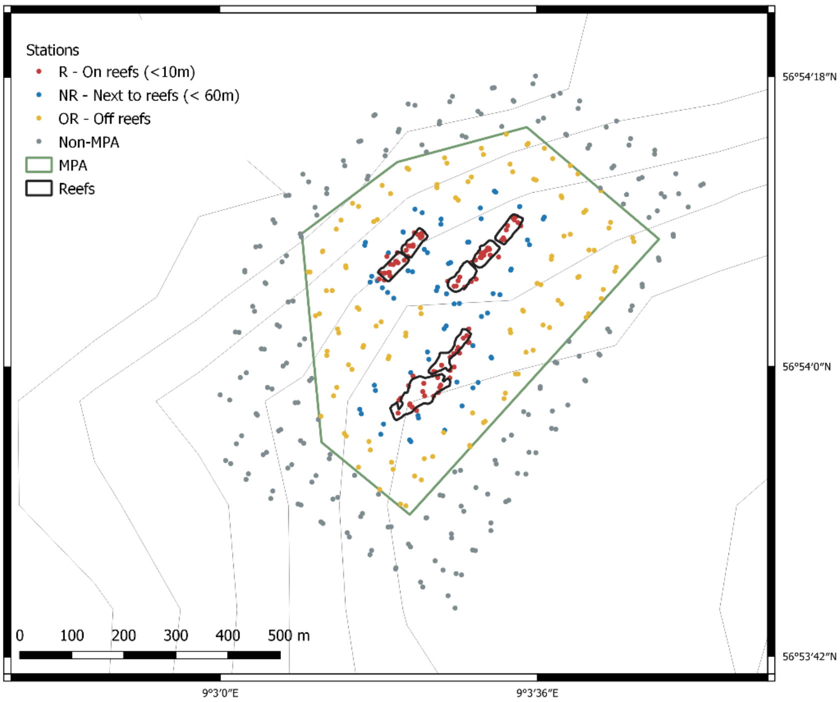
Formål med undersøgelserne er:

- Kortlægge forekomster af fisk og biodiversitet ved udlagte stenrev og i kontrolområder i Løgstør Bredning og sammenligne forekomsterne.
- Estimere bestanden af europæisk hummer på stenrevet i Løgstør bredning i relation til tæthed, fordeling, størrelse- og kønssammensætning samt reproduktiv kapacitet med henblik på at estimere revets betydning for bestanden af hummer.
- Estimere forekomst af strandkrabber på og udenfor revet.

1.3 Metoder

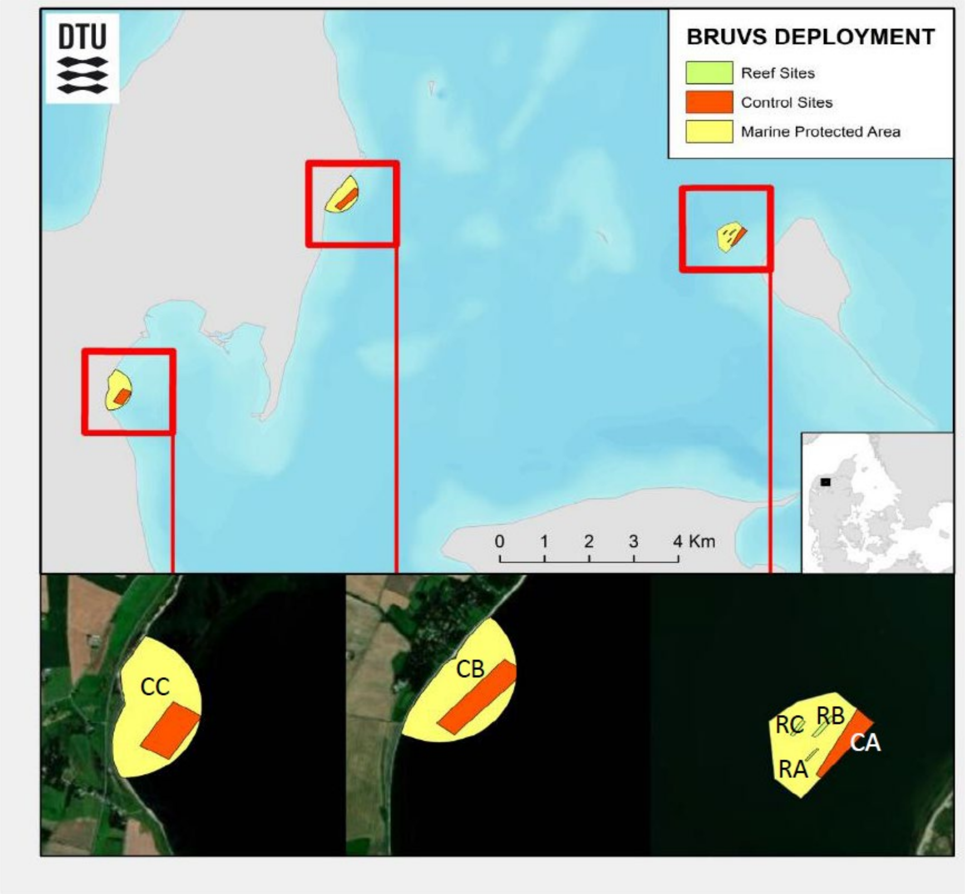
For begge de to undersøgelser gælder, at det ikke har været muligt at lave en undersøgelse med brug af et klassisk BACI-design (Before-After-Control-Impact), fordi der ikke er taget førprøver uanset undersøgelse. Derfor vil vurderingerne af effekten af stenrevet på den mobile fauna være baseret på forskelle mellem kontrolområde(r) og stenrevene i stenrevsområdet. I forhold til et control-impact design ville det endvidere være optimalt at kunne adskille effekter af selve stenrevet fra evt. effekter af det område, der er beskyttet mod fiskeri, og som er større end selve stenrevet. Det har imidlertid af forskellige årsager ikke været muligt indenfor projektets rammer med et sådant design for undersøgelser med brug af fiskerimetoder og effekterne er således målt som en samlet effekt af stenrevet og det tilhørende beskyttede område i sammenligning med et kontrolområde. Der er således for de to undersøgelser ikke valgt identiske kontrolområder. Alle prøver blev taget i sensommeren 2021 fra august til oktober.

For at kunne estimere forekomst og udbredelse af hummere, blev der designet en undersøgelse, der både vurderer hummerforekomsten og dens rumlige fordeling i et overordnet område i og omkring stenrevet som funktion af stenrevene, det beskyttede område, vanddybde og substrat. Der blev brugt følgende tekniker: i) Fangst-mærkning-genfangst (capture-mark-recapture, CMR), hvor hummer fanges og mærkes og efterfølgende genfanges med henblik på at få viden om deres fordeling og forekomst og ii) fangst pr. fiskeriindsats (catch per unit effort, CPUE), hvor der blev fisket med tejner på en række forskellige lokaliteter i og omkring stenrevet (figur 1). Alle hummer, der blev fanget i tejnerne blev talt, længdemålt og vejet og køn blev bestemt. Forekomst af strandkrabber blev alene bestemt via CPUE. De fangede strandkrabber blev talt og kønsbestemt og vejet som totalvægt fordelt på køn. Det skal bemærkes, at hummertejner ikke er optimale redskaber til fangst af strandkrabber, fordi maskestørrelsen generelt er stor i forhold til størrelsen af strandkrabber, og fordi der er relativt store indgangsåbninger, som strandkrabberne kan flygte ud af. Estimaterne af strandkrabber med denne metode er derfor semi-kvantitative. Vanddybderne i området blev bestemt fra sonar-målinger og søkort, og der blev brugt videooptagelser til at karakterisere bundforholdene. Yderligere og mere detaljeret beskrivelse af metoden kan findes i afsnittet ”An evaluation of the effects of the Livø stone reef and associated protected area on its lobster population”.



Figur 1. Prøvetagningsstationer på stenrevet (R, <10 m fra revet), ved siden af stenrevet (NR, >10, men <60 m fra stenrevet), udenfor stenrevet men inden for det beskyttede områder (OR) og uden for det beskyttede område (non-MPA). Dybdekurver (1 m) er vist. Indenfor det grønne område er der fiskeriforbundszone med stationerne R, NR og OR. Grå stationer ligger udenfor det beskyttede område.

Til at estimere forekomst af fisk og generel mobil fauna blev der brugt video-overvågning med kameraer påmonteret rammer, hvor der ligeledes blev monteret madding (BRUVS, Baited Remote Underwater Video Systems). BRUVS-systemet blev nedsænket og efter 5 minutter, hvor udstyrets position blev stabil og evt. resuspenderet materiale forsvandt, blev der lavet videooptagelser i 60 minutter. For hver nedsænkning blev maddingen skiftet for at sikre ensartede betingelser. Der blev foretaget videooptagelser på 6 lokaliteter – 3 indenfor stenrevsområdet og i 3 forskellige beskyttede kontrolområder. De 3 kontrolområder er valgt, så de matcher det nye stenrev med hensyn til vanddybde, substrattype (på nær forekomst af stenrev) og fiskeribeskyttelse (se figur 2). Alle videooptagelser blev analyseret med specialudviklet software til identifikation af især mobil fauna som krebsdyr og fisk. Større fastsiddende organismer som søanemone blev også dokumenteret. For alle organismer blev der gennemført identifikation af art (eller nærmeste artsgruppe) og beregnet MaxN. MaxN beskriver det maksimale antal individer (set på én gang) af en given art i løbet af 60 minutter og er det mest objektive mål for forekomst. Derudover blev dybden på lokaliteten bestemt, og der blev foretaget estimater af dækning af makroalger og blåmuslinger. På videooptagelserne blev kropslængde af den mobile fauna bestemt ved hjælp af en specialudviklet metode for de optagelser, hvor dyret blev optaget af begge kameraer i BRUVS stereokamera-opsætningen. Yderligere og mere detaljeret beskrivelse af metoden findes i afsnittet ”The effects of the Løgstør Bredning stone reef on fish and biodiversity”.

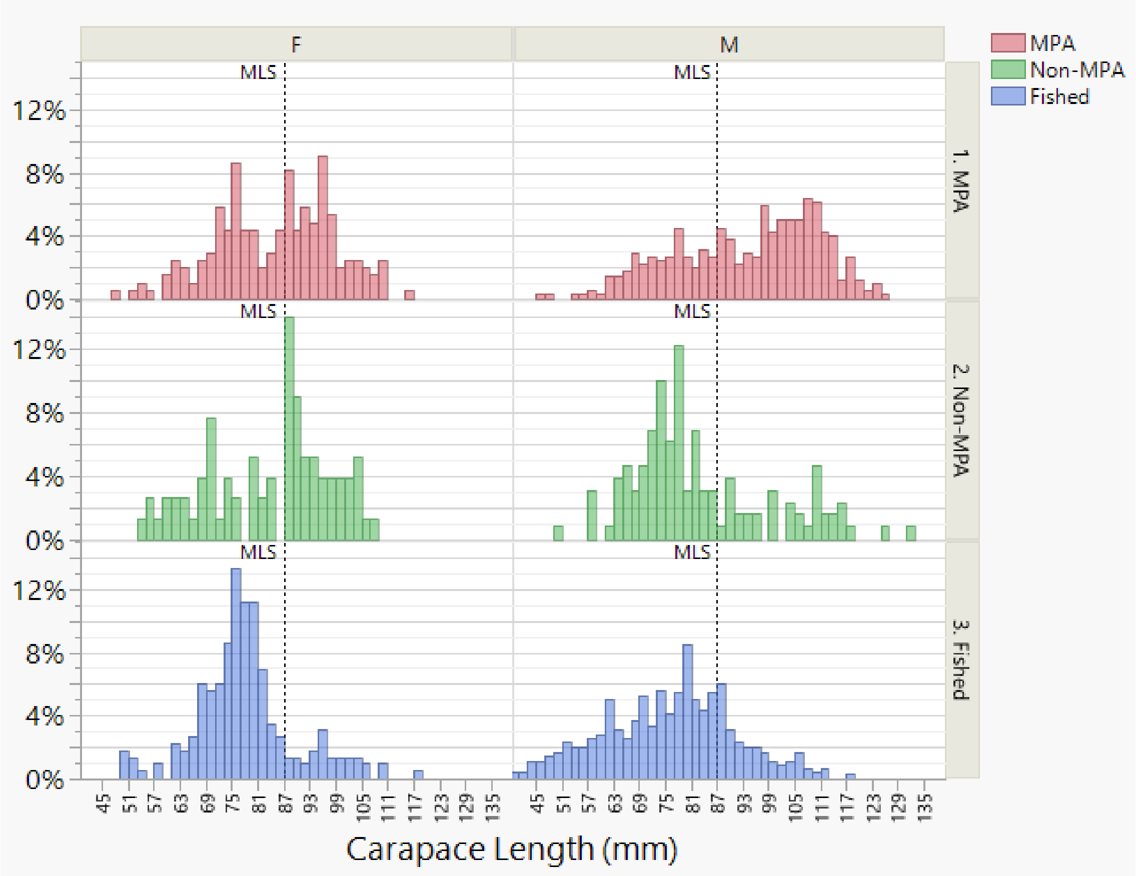


Figur 2. Prøvetagningsstationer for den mobile epifauna med brug af BRUVS-systemet med videooptagelser. Kameraer blev sat ud i kontrolområder CA, CB og CC (rød) og revområder RA, RB og RC (grøn). Alle BRUVS-udsættelser blev placeret inden for beskyttede havområder (gul).

1.4 Resultater

1.4.1 Hummer

Hummerbestanden i det beskyttede område (0.30 km^2) blev estimeret til 2.827±808 hummer sammenlignet med 1.326±799 hummer i det tilstødende kontrolområde (non-MPA i figur 1, 0.29 km^2) ved brug af CMR-metoden. Større tæthed af hummer (9,5±2,7 hummere pr. 1000 m^2) og fangstrate (CPUE; 0,833 hummere/tejne/dag) understøtter konklusionen, at der er flere hummer i det beskyttede område sammenlignet med det tilstødende ikke-beskyttede område (4,6 ±1,8 hummer pr. 1000 m^2 og 0,167 hummer/tejne/dag). CMR-forsøgene viste endvidere, at der var en betydelig migration af hummer ud af det beskyttede område. Den gennemsnitlige størrelse af de fangede hummer var ens indenfor det beskyttede område, det vil sige både selve stenrevet og det beskyttede område omkring stenene, men statistisk signifikant større end i det tilstødende ikke beskyttede kontrolområde (figur 3). Undersøgelserne dokumenterede også, at hummernes reproduktive potentiale i det beskyttede område er det samme som i det fire gange større kontrolområde. Dermed er der betydelige muligheder for positiv afsmitning af revet og det beskyttede område på bestanden i det omkringliggende område.



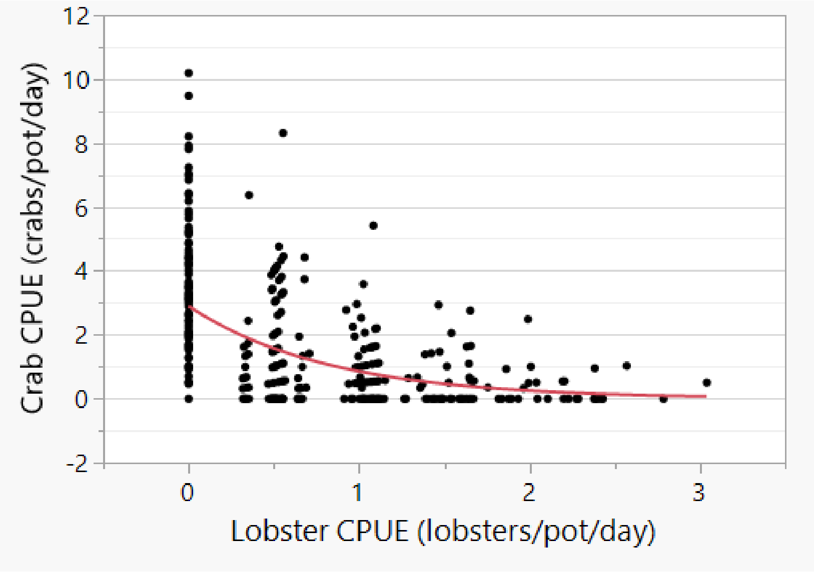
Figur 3. Histogrammer af hummer rygskjoldlængde (mm) for hunner (F) og hanner (M) i det beskyttede område (MPA, rød), det tilstødende ikke-MPA (grøn) og kontrolfisket sted (blå). Stiplet sort linje er minimum landingsstørrelse på 87 mm længde af rygskjoldet (MLS).

Analyser af BRUVS-data viste samme tendens. Forholdet mellem $MaxN_{rev}$ and $MaxN_{kontrol}$ var 6,5:1, det vil sige 6,5 gange flere hummere i det område med de nye stenrev sammenlignet med kontrol-områder uden stenrev. Forskellen mellem kontrol-områder og rev-områder var statistisk signifikant. Kontrol-områder og rev-områder var underlagt samme fiskerimæssige beskyttelse, således, at forskellen mellem områderne sandsynligvis primært skyldes tilstedeværelsen af stenrev.

1.4.2 Strandkrabber

Data fra videooptagelser med BRUVS viste, at forholdet mellem $MaxN_{rev}$ and $MaxN_{kontrol}$ var 0,5:1, altså dobbelt så mange strandkrabber i kontrolområdet som ved de nye stenrev, hvilket er den modsatte tendens sammenlignet med forekomsten af hummer. Fangst af strandkrabber pr fiskeriindsats (CPUE) var ligeledes signifikant lavere i det beskyttede område sammenlignet med kontrolområdet. Selvom de anvendte tejner ikke er optimale redskaber til strandkrabber, så kan det antages, at resultatet er retvisende, da det giver samme overordnede resultat som BRUVS-undersøgelsen.

Af CPUE-data fremgår det, at der kan være to forklaringer på forskellen i forekomst af henholdsvis strandkrabber og hummer på stenrevet. Der var således en signifikant forskel i CPUE af strandkrabber som funktion af substrattype med signifikant lavere forekomster på selve stenrevet sammenlignet med de andre substrattyper i områder. Der var desuden en signifikant negativ sammenhæng mellem forekomst af strandkrabber og hummer i tejnerne (se figur 5). Om det skyldes, at de to arter lever i forskellige habitater eller om forekomst af hummer i tejnerne eller i området skræmmer strandkrabberne væk, kan ikke afgøres af denne undersøgelse, men sammenholdt med de øvrige resultater, understøtter undersøgelserne, at de to arter foretrækker forskellige habitater. Analyse af video-optagelserne fra BRUVS-systemerne viste, at strandkrabber ofte trak sig tilbage, når en hummer dukkede op. Det tyder på, at hummer dominerer rev-habitatet.

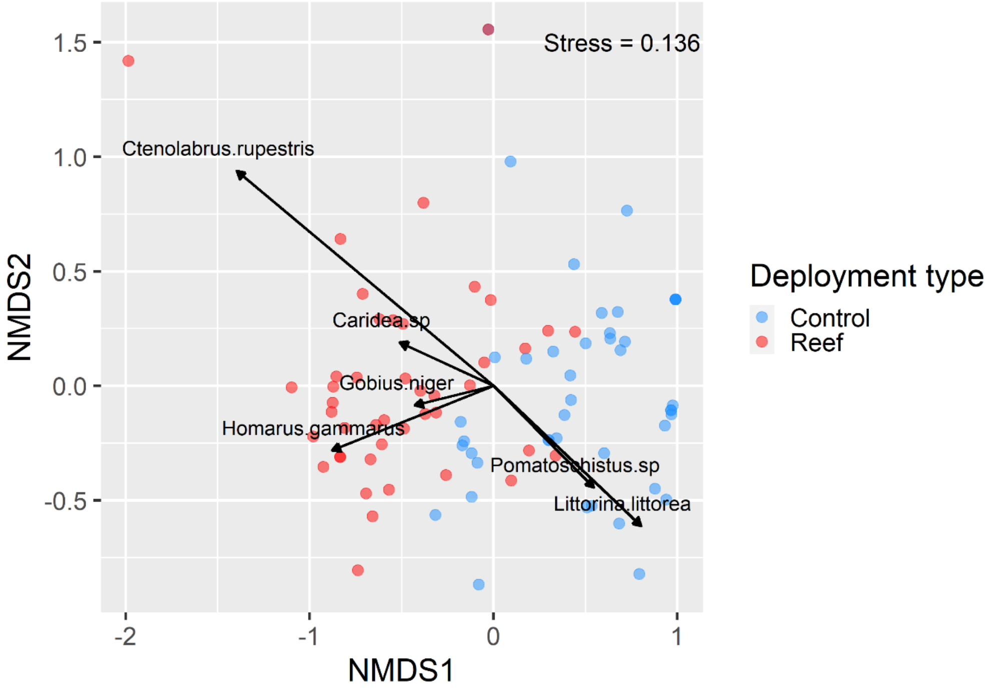


Figur 4. Regression mellem CPUE data (antal/tejne/dag) for strandkrabber som funktion af CPUE data for hummer.

1.4.3 Generel biodiversitet af mobile arter

Der blev i alt fundet 23 forskellige arter på BRUVS-optagelserne, hvoraf 11 er fiskearter som f.eks. havkarusse, sild og flere arter af kutlinger samt en del forskellige krebsdyrarter som hummer, strandkrabber og forskellige rejearter. Af de fundne arter er 3 dog gopler, som ikke selvstændigt kan bevæge sig uafhængigt af strømmen og derfor sandsynligvis ikke er påvirket af forekomsten af stenrev. Disse samt en søanemone art indgår ikke i de statistiske analyser.

Samlet set viser analyserne, at der er forskel på samfundene i henholdsvis rev-områderne og kontrolområderne. Ifølge BRUVS-optagelserne var der 23 forskellige arter i optagelserne, hvoraf 17 arter forekom i både kontrol- og rev-områder. Der var imidlertid signifikant forskel i antal arter i de enkelte BRUV-optagelser mellem rev-områderne og kontrol-områderne. Ligeledes var Shannon-Wiener og Pielous Evenness-indeks både statistisk signifikante og i gennemsnit højere for rev-områderne. De to indeks indikerer henholdsvis højere diversitet og mere jævne forekomster af forskellige organismer. Resultaterne viser, at samfundene i rev-områderne og kontrol-områderne ofte består af forskellige arter, der også kan variere i forekomsterne. For eksempel var der ofte større forekomster af europæiske hummer og forskellige rejer i rev-områderne. En relativt høj værdi af Evenness indikerer normalt, at samfund er mere homogene eller ensartede (Smith et al., 1996), og derfor kan den lavere Pielous Evenness i kontrol-områderne delvist afspejle et samfund domineret af strandkrabber, i modsætningen til revene som er domineret af flere arter som havkarusse, hummer, sortkutling og forskellige rejerarter. En multivariat analyse af arts-sammensætning i rev- og kontrol-områder viser ligeledes, at der er tale om forskellige samfund i de to forskellige habitater (Figur 5). Der er således høj forekomst af hummer og rejer samt fiskearter som havkarusse og sortkutling i rev-områder, mens strandkrabber og arter som sandkutling og alm. strandsnegl ofte dominerer i kontrol-områderne.



Figur 5. Resultater af non-metrisk multidimensionel skalering (nMDS) af Bray-Curtis ulighed i artssammensætning. Farverne skelner mellem rev-områder (rød) og kontrol-områder (blå). Pile repræsenterer arter, der er signifikant korreleret med specifikke lokaliteter (rev- og kontrolområder). Pilenes længde angiver virkningen af hver enkelt art. Analysen viser forskellige biologiske samfund i rev-områder og kontrol-områder.

1.5 Konklusioner og diskussion

Det kan overordnet konkluderes, at de nyetablerede stenrev ved Livø har haft en betydning for forekomst af mobile faunaarter som fisk og krebsdyr. Dette er i overensstemmelse med undersøgelser af den mere fastsiddende fauna. Der er en tydelig forskel mellem stenrevet samt dets nærmeste omgivelse indenfor det beskyttede område og de valgte kontrolområder. Fordi der ikke er data for mobile faunaarter, før stenrevet blev anlagt, er konklusionerne ikke fuldstændigt entydige, men det er helt overvejende sandsynligt, at de observerede forskelle mellem kontrolområder og det beskyttede område er udtryk for en effekt af det anlagte stenrev og den tilknyttede beskyttelse. Dette understøttes både af studier fra andre områder med nyetablerede stenrev og for den videnskabelige litteratur om de forskellige arters foretrukne habitater. De overordnede forskelle i diversitet af den mobile fauna mellem kontrol-områder og revene er drevet af arter som havkarusse, hummer og rejer på revene og strandkrabber og kutlingearter udenfor revene. Derudover bidrager forskelle i mindre fisk til forskellene mellem områderne. Dermed kan det også konstateres, at selv i stærkt belastede områder som Limfjorden, kan genetablering af habitater lede til forandringer og variationer i det associerede dyre- og planteliv og bidrage til en øget lokal biodiversitet.

Det er bemærkelsesværdigt, at der stort set ikke optræder kommercielt relevante fiskearter i optagelserne på nær sild. Torsk, fladfisk og lignende arter er fåtallige eller totalt fraværende. Sild optræder i undersøgelserne og der er et kommercielt fiskeri af sild og i nogle år brisling i Limfjorden. Disse arter er antaget at være indvandret fra Nordsøen eller Kattegat. Der er flere mulige forklaringer på fravær eller meget lave forekomster af kommercielt interessante fiskearter på nær sild og brisling, som f.eks. Limfjordens generelle miljøtilstand, fiskeritryk udenfor fjorden, historisk fiskeritryk i fjorden, forekomst af prædatorer som skarv og spættet sæl mm. Det er ikke muligt indenfor rammerne af dette studie at udrede dette. Undersøgelserne viser dog, at indenfor en kort tidshorisont vil etablering af egnede habitater som beskyttede stenrev ikke i sig selv bidrage til at øge forekomsterne af fraværende arter i Limfjorden.

Der er anvendt forskellige metoder og forskellige kontrolområder i det samlede studie. Det gør, at der ikke kan foretages en direkte kvantitativ sammenligning mellem resultaterne af de to studier, dvs. studiet med fangst i tejner og studiet med BRUVs. Der er også forskelle mellem arterne i undersøgelsen i forhold til, om de udnyttes kommercielt. Der er som den eneste krebsdyrart et fiskeri på hummer i Limfjorden, og bestanden kan forventes at være påvirket af fiskeritrykket. Selvom undersøgelserne med tejner blev gennemført i den periode, hvor hummerfiskeriet er lukket, blev der alligevel observeret en del hummertejner i området. Det skyldes, at det udelukkende er forbudt at lande hummer i den lukkede periode, ikke at have redskaber stående, der potentielt/teoretisk kan fiske efter andre arter som taskekrabber eller fisk.

Der er et ganske omfattende kommercielt og rekreativt fiskeri af hummer i Limfjorden. Det kan derfor være værd at bemærke, at stenrevet og det dertil hørende beskyttede område har en effekt på bestanden af hummer i det beskyttede område. Der er således en signifikant større forekomst af hummer i det beskyttede område, den individuelle størrelse af hummer er større i det beskyttede område, og der er et større reproduktivt og gydemæssigt potentiale i det beskyttede område. Det betyder, at stenrevet og det beskyttede område i kraft af hummernes vandring har en positiv effekt på bestanden udenfor revet, og det kan derfor overvejes at fastholde beskyttelsen af området. På den anden side vil fiskeritrykket udenfor det beskyttede område også kunne påvirke hummerbestanden på revet og i det beskyttede område. Det kan i den forbindelse overvejes om det beskyttede område har den rette størrelse eller med fordel kan gøres større. Der er ingen data, der konkret kan understøtte, hvad den langsigtede effekt af en fredning kan være, da der generelt set ikke er foretaget overvågning af bestanden af hummer i Limfjorden.

Hvorvidt beskyttelse af andre lignende rev-områder i Limfjorden vil have samme effekter for de lokale forekomster af hummer kan ikke dokumenteres alene af dette studie, men lignende effekter kan antages i områder med stort fiskeritryk.

1.6 Litteraturhenvisninger

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2. An evaluation of the effects of the Livø stone reef and associated protected area on its lobster population

Pedro S. Freitas, Elliot J. Brown, Jon C. Svendsen, Trine G. Hansen, Jens Kjerulf Petersen

2.1 Summary

Artificial reefs and no-take marine protected areas can have a positive effect on European lobster (*Homarus gammarus*) population by providing high value and rare habitat (e.g. large boulders and shelters), as well as protection from fishing. The Livø artificial stone reefs (LSR) in the Limfjorden were established in 2017 to investigate its use as a supplementary planning tool for nutrient mitigation and assess its impact on macroflora and macrofauna biodiversity. A protected area (MPA) of ca. 0.3 km^2 surrounding the reefs and closed to fishing was created in 2018. Any effects of the LSR and its MPA on the lobster population and its status are currently unknown.

A study was undertaken in the summer 2021 to assess the status of the European lobster population in the LSR and MPA. The objectives of the study were: 1) An assessment of the abundance, distribution, size, sex and reproductive potential of the lobster population in the LSR-MPA; 2) A preliminary evaluation of potential effects on the lobster population from the rare reef habitat and fishing protection provided by the LSR-MPA and spill-over into adjacent non-protected areas (non-MPA) as evidenced e.g. from tagging-recapture experiments and size distributions in the respective areas; 3) An opportunistic non-quantitative evaluation of shore crab abundance in the LSR-MPA was also performed.

The size and distribution of the lobster population in the LSR-MPA and in an adjacent non-MPA site were studied using a capture-mark-recapture (CMR) method and pot-based surveys. The lobster population in the MPA was estimated at 2,827±808 lobsters in 0.30 km^2, while at the similar sized adjacent non-MPA was estimated at 1,326±799 lobsters in 0.29 km^2. Lobster density (9.5±2.7 lobsters per 1,000 m^2) and catch per unit effort (CPUE; 0.833 lobsters/pot/day) was thus twice as high in the MPA than in adjacent non-MPA (4.6±1.8 lobsters per 1,000 m^2 and 0.167 lobsters/pot/day). Lobster abundance decreased with increasing distance to reefs and the MPA boundary, reflecting a positive effect from reef habitat and/or decreasing protection from fishing mortality from the centre of the MPA toward its boundary. Male lobster abundance, but not of female lobsters, was higher in the stone reefs and immediately surrounding area than in non-reef areas of the MPA, suggesting a sex-specific habitat effect.

Lobster size was similar in reefs and off-reef areas within the MPA, but significantly larger in the MPA than in the control fished site. Only in the MPA was length at first capture (L_{95}), when 50% of the population is retained by fishing gear, larger than the minimum landing size (MLS) of 87 mm carapace length. In the MPA, both male and female lobsters reach significantly larger sizes

than in control fished site. However, only male lobsters were larger in the MPA than in the adjacent non-MPA suggesting that the MPA impact on survival and protection from fishing mortality is sex specific.

The combination of higher lobster abundance (CPUE) and larger size in the MPA results in significantly higher reproductive potential than in the control fished site. Egg production in the MPA, excluding the positive spill-over effect on female lobster abundance and size in adjacent areas to the MPA, is estimated to be equivalent to a fished area ca. 4 times larger in size.

Significant spill-over of lobsters from the MPA to surrounding areas was observed. Lobsters moved several hundred meters to over 1,000m within a few days to a few weeks, resulting in larger lobsters and higher reproductive potential in the non-MPA site adjacent to the MPA relative to the control fished site and in significant captures of lobsters originating from the MPA in fishing grounds surrounding the MPA. 3.0% of all tagged lobsters in the MPA, or 4.6% larger than minimum landing size, were fished by a single fisherman in the 4 weeks following the study, corresponding to landings of 71 lobsters or 91.9 kg obtained from the MPA.

Shore crab abundance was lower in the stone reefs and in the MPA than in adjacent areas outside the MPA, likely due to a combination of negative effects from reef habitat that is not favoured by shore crabs and agonistic interactions with lobsters, but not from MPA protection as crabs are not fished in the area.

In summary, the results obtained support a clear positive impact of the LSR-MPA on the lobster population after only 3 years since its implementation, resulting in a larger population and larger lobsters in the MPA, with spill-over effects onto adjacent areas. The findings from this study support the implementation or restoration of stone reef habitats and small MPAs as valid and significant tools in the conservation of lobster populations in the Limfjorden, which can also play a significant role in the restoration and conservation actions in other Danish coastal systems.

2.2 Introduction

Species with moderate mobility, high-site fidelity and exposure to high levels of fishing mortality, such as lobsters (e.g. Hoskins et al., 2011; Moland et al., 2011; Skerrit et al., 2015), have been shown to have the strongest responses to protection in MPAs (e.g. Halpern et al., 2004; Micheli et al. 2004, Goñi et al. 2010). Artificial stone reefs and MPAs can have positive impacts on European lobster (*Homarus gammarus*) populations by providing high value and rare habitat (e.g. large boulders and shelters), as well as protection from fishing (e.g. Jensen, 2000; Spanier et al., 2011; Moland et al., 2013a). Increases in European lobster abundance, size and reproductive potential have all been observed in MPAs relative to non-protected control areas (e.g. Hoskins et al., 2011; Moland et al., 2013b; Calef, 2016). In addition, MPAs can provide a spill-over effect into adjacent unprotected areas, through either the migration of juvenile and adult individuals or the export of larvae that can be beneficial for both conservation purposes and surrounding fisheries (e.g. Goñi et al., 2010). The effects of MPAs depend on multiple factors, such as its size, isolation, connectivity to other MPAs, protection enforcement, time since implementation, all of which often have opposing impacts on different conservation and spill-over management goals (e.g. Halpern et al. 2010; Edgar, 2014).

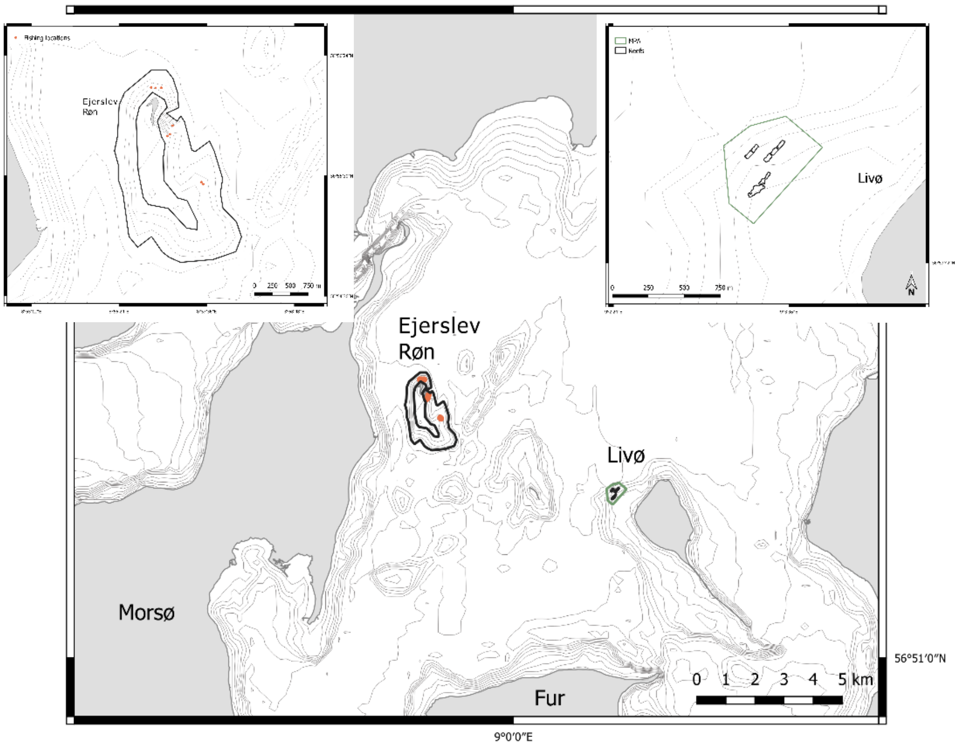

Figure 1. Location of the Livø stone reefs and marine protected area (top right) and control fished grounds in Ejerslev Røn (top left) in the Limfjorden. 1m bathymetric lines are shown.

The Livø artificial stone reefs (LSR) in the Limfjorden (Figure 2) were established in Autumn 2017 to investigate its use as a supplementary planning tool for nutrient mitigation, but also to assess its impact on local macroflora and macrofauna biodiversity, and to re-establish stone reefs (e.g. reports in https://www.stenrev.dk/rapporter-video-og-billeder/; Vedel, 2016). A temporary no-take zone (Marine Protected Area, MPA) surrounding the reefs of ca. 0.297 km^2 where all forms of fishing are forbidden was created in mid 2018 (BEK nr 786 af 08/06/2018). Seven separate reefs in three groups constitute the LSR (Figure 1): two western reefs and three central reefs made of Norwegian granite boulders and two eastern reefs are made of small, round fieldstones (Møhlenberg, 2016). The LSR and MPA are located northwest of Livø Island in the Limfjorden between 3 to 9 m water depth, on an area with hard substrate (Figure 2) that previously contained large rocks and stone reefs, which were exploited for stones over the past century (Vedel, 2016).

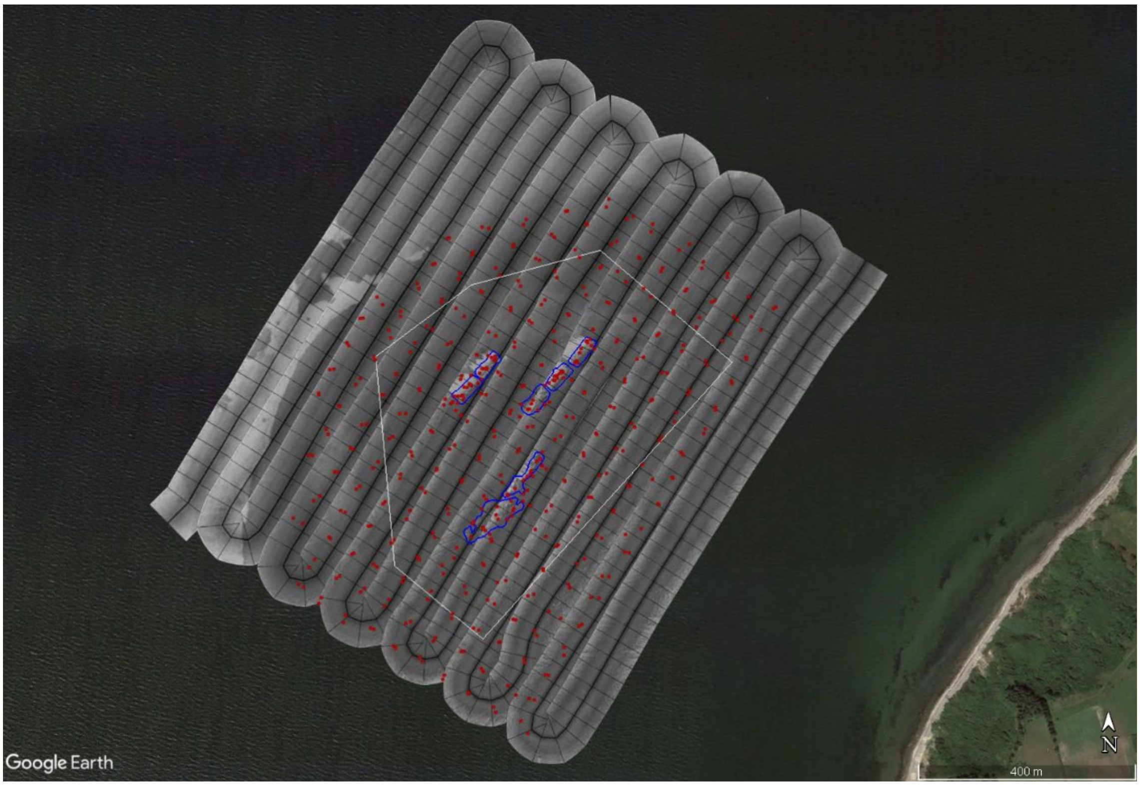

Figure 2. The stone reefs (blue lines) and marine protected area (white line) NW of Livø island. Background is a side-scan mosaic identifying the reefs and indicating bottom hardness (inverted scale, hard is dark and soft is lighter, except by the reef where light is a shadow effect). Red dots area sampling stations Image from Google Earth.

The Limfjorden lobster fishery is the main Danish coastal lobster fishery with ca. 22 tons/year and 73% of Danish landings since 2015. The current fishery started after 2005 following several decades with no or little landings and is now locally economically and culturally important as a mixed commercial and recreational fishery, albeit a data poor fishery (ICES Category 5).

Protection and restauration of lobsters is in accordance with the original purposes for the establishment of the LSR and MPA. Evidence of a short-term impact by the implementation of the LSR on lobsters was observed by the rapid increase of fishing gear in the LSR, which lead to the establishment of the temporary no-take zone MPA. However, the effects of LSR and MPA on the lobster population, as well as its interactions with adjacent non-protected areas and its relevance for the Limfjorden lobster population and fishery are unknown and form the rationale behind the present study.

The objectives of the study were:

a. An assessment of the abundance, distribution, size, sex and reproductive potential of the lobster population in the LSR-MPA.
b. A preliminary evaluation of potential effects on the lobster population from the rare reef habitat and fishing protection provided by the LSR-MPA and spill-over into adjacent non-MPA.
c. An opportunistic non-quantitative evaluation of shore crab abundance in the LSR-MPA, profiting from the lobster survey.

This report presents results for capture-mark-recapture estimates of lobster population size, lobster spatial distribution, size distribution and sex structure, reproductive potential, and shore crab distribution in and around the LSR-MPA, as well as an initial assessment of spill-over effects of the LSR-MPA based on lobster abundance, mobility and size distribution.

2.3 Survey Design and Methods

To provide unambiguous evaluation and separation of reef habitat and MPA protection effects on the lobster population, a BACI before-after-control-impact approach would be required to disentangle impact effects from spatial and temporal variations (e.g. Hoskins et al., 2011; Moland et al., 2013a,b). For instance, the reefs and MPA could have higher lobster abundance than the adjacent non-MPA site or a distant control area before the implementation of the reefs and MPA. Eventually, a type I error could occur, false rejection of a true null hypothesis: e.g. observing a difference between the reefs and MPA relative to control sites and reject that lobster abundance in reef and non-reef areas and in MPA and non-MPA areas was equal and unchanged by the establishment of the reefs and MPA. However, the inexistence of prior knowledge to the implementation of the LSR-MPA on the lobster population excludes before-after designs.

A control-impact design requires control sites similar in size and environmental setting to the Livø MPA. True control areas in a control-impact design to disentangle the effects of the reefs and MPA in the lobster population would be fished areas away from the MPA with and without reefs, as well as an MPA without reefs, all with similar substrates and depths as the Livø MPA. With the sampling time and resources available, such approaches were not feasible and could not be fully implemented. Therefore, a “partial” control-impact design was adopted with the artificial reefs and MPA as impact sites and controls sites in off-reef areas, the adjacent area surrounding the MPA (non-MPA), and for size and reproductive potential a true distant control fished site (Ejerslev Røn).

However, the off-reef and non-MPA areas are not true controls since they are not independent from the impact sites and are affected by the MPA and reefs due to its proximity. Considering the small distance from the reefs to the MPA boundary (mean of 163±3.9 m SE) and the relatively small size of the Livø MPA (0.3 km^2), lobsters can move from the reefs and/or the MPA into adjacent areas and vice-versa versa (e.g. Skerrit et al., 2015; Moland et al., 2011) and were actually observed to do so in this study. Therefore, adjacent off-reef and non-MPA sites are expected to have higher lobster abundance and size than true unaffected control areas would have, as observed elsewhere in other MPAs (Hoskins et al., 2011; Moland et al., 2011; Huserbråten et al., 2013; Thorbjørsen et al., 2018; Kleiven et al. 2019). Similarly, fishing mortality in nearby non-MPA areas will negatively impact lobster abundance inside the MPA, including the reefs. The consequence of the limitations described above is a reduced discriminant power (not for size and reproductive potential) and underestimation of impacts from the reefs and MPA, as the amplitude of differences in density and abundance between impact (i.e. reefs and MPA) and control sites (i.e. non-MPA) is reduced. Nevertheless, such approach allows a preliminary and valid assessment of effects from the artificial reefs and MPA on lobsters.

The study consisted of a capture-mark-recapture study to estimate absolute lobster population, combined with a survey of lobster distribution in the MPA and adjacent non-MPA. Size and sex structure, and reproductive potential were additionally compared to a true control fished site.

2.3.1 Survey design

To assess the abundance and distribution of lobsters, a dual-purpose survey in and around the LSR-MPA was designed to: i) estimate lobster abundance from capture-mark-recapture (CMR) and catch per unit effort (CPUE); and ii) assess lobster spatial distribution relative to the stone reefs and MPA, depth and substrate. The two purposes have somewhat different requirements. CMR requires at least two surveys, one to mark and a second to recapture marked lobsters, covering the area occupied by the population, as well as sufficient catches to mark a significant fraction of the lobster population and ensure a high enough recapture rate. Therefore, CMR is favoured by a dense, regular coverage of a smaller area, while spatial distribution surveys benefit from random coverage of a larger area over a wider range of environmental variables.

To accommodate the requirements of both CMR and spatial distribution assessments, two surveys were conducted using stratified sampling on a 55 m regular grid in non-reef areas and randomly in each of the reefs (Figure 3). The design assumed a mean catch rate of 0.19 lobster per pot per day as observed in and around the MPA in a 2020 survey (unpublished, project “Bæredygtigt hummerfiskeri i Limfjorden”; EMFF, 33113-B-19-137), significantly lower than obtained in this study (see Section 5). In total, an area of 0.59 km^2 was sampled, with the reefs and MPA occupying ca. 0.30 km^2 at its centre surrounded by a fished non-protected area of ca. 0.29 km^2, non-MPA (Figure 3). The surveys were performed during the closed fishing season in July and August from 28/07/2021 to 08/08/2021 and from 17/08/2021 to 02/09/2021. It must be noted that fishing gear was observed in the vicinity of the MPA from the 10/08/2021.

Each survey consisted of five hauling days, using 50 single lobster pots (double entrance, single parlour, Carapax) baited with herring and cat food, randomly distributed on a regular grid and on the three reef groups. Pots were moved to new locations each hauling day. A short two-day soak time was planned to avoid pot saturation. However, bad weather conditions during both surveys resulted in variable soak times of 2 days (191+49 pots), 3 days (50+141 pots), 5 days (9 pots) and 6 days (50 pots) and a 9-day interval between surveys. In total, survey A sampled 135 stations in the MPA, 36 of which on the reefs, and 106 outside the MPA, while survey B sampled 145 stations in the MPA, 36 of which on the reefs, and 104 stations outside the MPA.

Stations were classified in three categories based on its position relative to the reefs and MPA (Figure 3): on reef (R) if less than 10m from the reefs boundaries to accommodate uncertainties in the location of the pots and reef boundaries; next to reef (NR) from 10 to less than 60m from the reef boundaries to reflect the immediate vicinity of the reefs; and off-reef (OR) if more than 60m from the reef boundaries; inside (MPA) or outside the MPA (non-MPA)

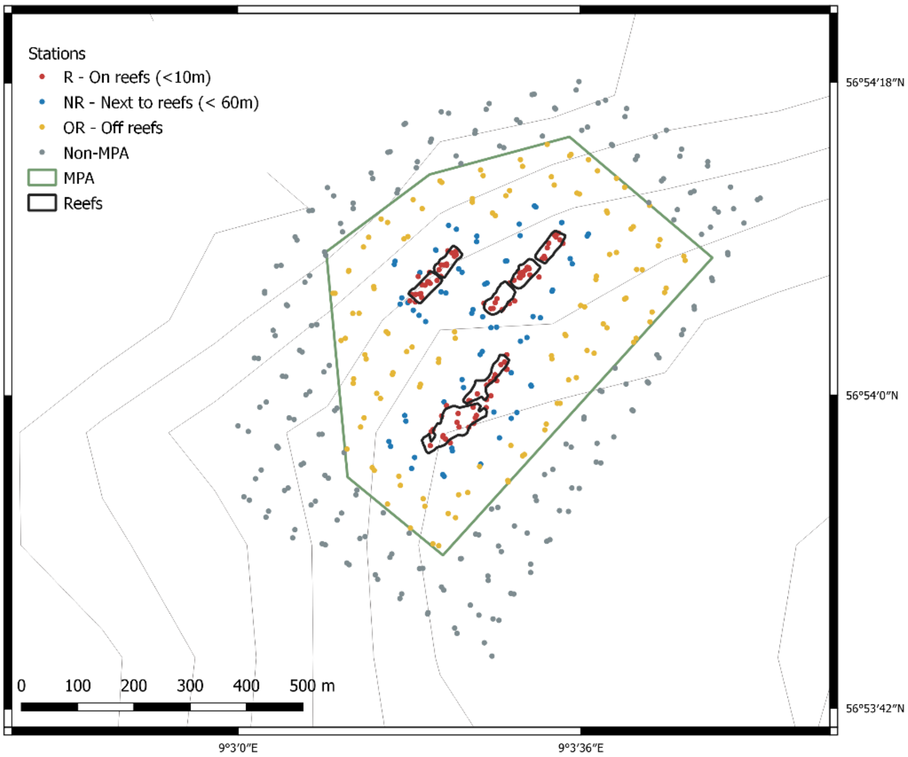

Figure 3. Sampling stations inside the MPA (green line): the reefs (R, < 10m from reef boundaries, red dots), next to reef (NR < 60m from reef boundaries, blue dots) and off-reef (OR, yellow dots). Stations in the adjacent fished non-MPA (grey dots) outside the MPA. 1m bathymetric lines are shown.

Upon capture all lobsters were counted, measured, weighed, sex and egg-bearing status assessed, tagged with individually numbered T-bar tags (Hallprint) in the dorsal musculature between the cephalothorax and abdomen, and a V-notch was made in a tail uropod (Figure 4). Lobsters were then released at their capture location.

Weather permitting, shore crabs were sorted counted and weighed per sex. However, lobster pots are not appropriate for shore crab sampling, with too wide net mesh size and large apertures that allow easy exit of shore crabs. At best, only semi-quantitative catch data of shore crab is obtained.

Figure 4. A lobster marked with T-bar tag (white circle) and V-notch (red circle) in a tail uropod.

2.3.2 Capture-Mark-Recapture

The size of the lobster population was estimated using the Chapman adaptation of the Lincoln-Petersen method for single marking and single recapture, which reduces bias at small sample sizes (Chapman, 1951):

$$\mathrm{N} = \frac{(\mathrm{K}+1)(\mathrm{n}+1)}{(\mathrm{k}+1)} - 1$$

Where N is the estimated number of animals in the population, n is number of animals marked in first visit, K is the number of animals captured in the second visit and k is the number of re-captured marked animals.

Confidence limits were calculated from variance estimates as 95% confidence limits = N± 1.96√V, V = variance (Hart and Gorfine, 1997):

$$\mathrm{Variance\ (N)} = \frac{(\mathrm{K}+1)(\mathrm{n}+1)(\mathrm{K}-\mathrm{k})(\mathrm{n}-\mathrm{k})}{(\mathrm{k}+1)(\mathrm{k}+1)(\mathrm{k}+2)}$$

The estimation relies on several assumptions and is considered a rough estimate, but allows abundance estimation in small, short studies when only single marking and single recapture is possible. Method assumptions are: 1) the surveyed area is a closed system, with no change in number animals in the population from migration, mortality or natality. 2) the probability of being captured and recaptured is the same for all animals in the populations and is not affected by tagging or marking; 3) tags or marks are not lost between marking and recapture surveys.

Several lobsters recaptured in the second survey had lost the T-bar tags (17.5%) but were identified from the V-notches. A small proportion of tagged lobsters were recaptured in the same survey of marking, 7 and 10 lobsters or 0.16 and 0.17% in Survey A and B, respectively, and were excluded from population estimates.

Both surveys caught similar proportions of female and male lobsters that differed only by 2%, and the mean sex ratio of both surveys (34.1% females to 65.9% males, N = 840) was used to estimate female and male lobster populations.

2.3.3 Mobility

The marking and recapture positions of lobsters during CMR surveys A and B, together with fishermen capture reports of tagged lobsters (tag ID, location and time of capture) in the four weeks following the CMR surveys were used assess lobster movement. Movement distance and azimuth were determined using QGIS software.

2.3.4 Catches and spatial distribution

High variability was observed in catch per pot relative to soak time (Table 1), and fishing efficiency of pots can be expected to be reduced by gear saturation effects at long soak times (e.g. Bennett and Lovewell, 1977; Groeneveld et al., 2003). Gear saturation deviates the relationship between catch and soak time from the expected linear increase, where catch increase per soak day unit equals the catch at one soak day (Table 1). Catch data with 5 and 6 soak days were thus excluded from catch per unit effort and distribution analysis (CPUE, expressed as lobsters per pot per day). CPUE is as an indirect proxy/indicator of population abundance.

Table 1. Mean catch (lobster per pot haul) per soak days in Survey A and B estimated from zero Inflated Poisson or Poisson* distributions. SE is standard error.

	Survey A			Survey B		
Soak Days	N	Mean catch	SE	N	Mean catch	SE
2	191	1.99	0.14	98	1.38	0.18
3	41	2.56	0.31	101	2.25	0.19
*5	9	3.44	0.62			
6				50	2.76	0.27

2.3.5 Environmental variables

Possible explanatory variables of lobster distribution were obtained for each station. Depth was obtained from navigation chart or from sonar measurements on the reefs (chart depth not available), while video footage of the bottom was used to classify the substrate as (Figure 5): hard-boulders (H), gravel (G), mixed gravel and sand (M) and sand (S). The boundary limits of the MPA (https://www.retsinformation.dk/api/pdf/201871) and reef structures (obtained from side-scan sonar) were used to classify each station as reef (R), next to reef (NR) and off-reef (OR) as described previously, and as inside (MPA) or outside the MPA (non-MPA; Figure 3). The minimum distance from each station to the MPA and reef structure boundaries was determined using QGIS.

2.3.6 Lobster distribution modelling

Lobster spatial distribution was modelled using a general linear mixed model with number of lobsters at each site as the response variable offset by logarithmic soak time to account for variable sampling effort. Sampling day was included as a random variable and the explanatory variables were depth, bottom substrate and distance to reef. As the response variable is count data, a Poisson and two negative binomial probability distributions, with variance increasing linearly (nbinom1) or quadratically with the mean (nbinom2), were tested. Evaluation of residuals, zero inflation and the degree of dispersion indicate lobster abundance to be best described by nbinom1 distribution. Different explanatory variables were tested, and the most parsimonious model was selected using the Akaike information criterion corrected for small sample sizes (AICc).

For model validation, data was split into training and testing data sets, with 90% of data used for training and re-estimating model parameters, and the remaining 10% of data used to test the new fits in 50 iterations. Bias, root mean square error (RMSE), mean absolute error (MAE), and R-squared (Rsq) were used to assess model fit. All modelling was done using R Studio and glmmTMB, DHARMa and Caret packages.

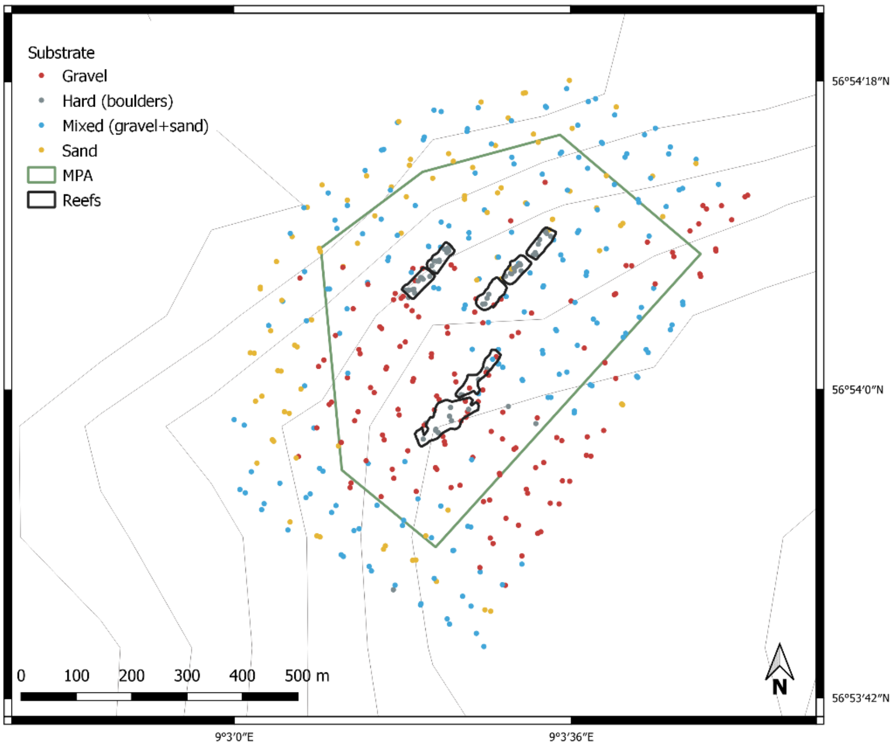

Figure 5. Sampling stations according to substrate: hard-boulders (H, grey dots), gravel (G, red), mixed gravel-sand (M, blue) and sand (S, yellow).

2.3.7 Size measurements

Carapace length (CL) was measured to the nearest mm from the eye socket to the posterior edge of the cephalothorax (N = 275) or estimated from the distance (CR) from the tip of the rostrum to the posterior edge of the cephalothorax using a linear regression (N = 531): CL = 0.8319*CR - 4.211, N = 266, RMSE = 2.04, $r^2 = 0.986$, $p < 0.0001$. Lobsters were sexed based on morphological differences in the first pair of pleopods/swimmerets and the egg bearing status of females determined.

Lobster size in the MPA was compared to a control non-protected fished ground in Ejerslev Røn, off NE Morsø (Figure 1; unpublished, project "Bæredygtigt hummerfiskeri i Limfjorden"; EMFF, 33113-B-19-137). A set of carapace length catch data from September and the first week of October 2021 was provided by three recreational fishermen using 18 multipots (kinaruser, 10 m length, 18 entrances, no escape vents). Since fishermen gear was different than the lobster pots used in the CMR surveys, a calibration factor of 4.62 (3.0 to 7.9) pots per one multipot was used to convert fishermen multipot CPUE data (Frandsen and Feekings unpublished, Hummerfiskeri project EMFF). Both types of gear had no escape vents and are expected to capture similar sizes.

Logistic fits to the cumulative proportion of catches per lobster size (CL in mm) were used to estimate two size -based indicators that describe the length frequency distributions of catches and are used in length-based fisheries management. Size at first capture (L_C), the length at which 50% of the population is retained by the fishing gear. Length at which 95% of the population is retained by the fishing gear (L_{95C}), an indicator for the presence of the largest length groups in the catch. L_{95C} was chosen instead of maximum length (L_{max}) or mean length of largest 5% of catch (L_{max5}), due to the rarity of large individuals above quantile 95% in non-MPA (N=10) and fished ground (N = 5). Logistic fits to the cumulative proportion of legal catches per lobster size larger than MLS (i.e. potential landings) were used to estimate size where catches would reach at 50% (L_{50}) and 95% (L_{95}) of total landings. Logistic models followed:

$$\% \, Catch = \frac{1}{1 + e^{-a*(CL-b)}}$$

Where % Catch is the proportion of harvestable catch, CL is carapace length, a and b are model parameters: a is growth rate and b is inflection point (Table 2).

Table 2. Logistic models parameters by area and size distribution indicator.

Marking Area		L_C+ L_{95C}	L_{50}+ L_{95}
MPA			
	a	0.098	0.166
	b	89.879	100.508
Non-MPA			
	a	0.108	0.150
	b	81.905	97.227
Control Fished			
	a	0.126	0.186
	b	75.888	93.217

2.3.8 Reproductive potential and spawning indices

An index of relative reproductive potential (RRP) of each 3 mm size class was calculated as (Tully et al., 2001 and references there in):

$$RRP = Pm_i * F_i * N_i$$

Where Pm_i is the proportion of functionally mature lobsters (i.e. ovigerous) in size class *i*, F_i is the fecundity of size class *i* and N_i is the number of lobsters in size class *i* expressed as the percentage of total sample size. The sum of RRP per size class produces an index of reproductive potential for each area.

In our study, RRP reflects only differences due to female size distribution, since Pm_i was assumed to be the same in all areas, but it does not consider differences in population size. Lobster functional maturity, and thus P_m, should be determined in spring prior to the spawning and hatching periods in summer (Tully et al., 2001; Agnalt, 2007; ICES, 2003). Due to the time of data collection from mid-summer to early autumn and small sample size, P_m was estimated from catch report data obtained in the springs of 2020 and 2021 from several fishing grounds across the Limfjorden (unpublished, Hummerfiskeri project EMFF: 33113-B-19-137). The proportion of mature females at any given size is thus assumed to be the same across the Limfjorden. A binomial logistic function was fitted to the relationship between the proportional functional maturity (P_{mi}, ovigerous ratio) and carapace length (CL_i) per 3 mm size classes (Figure 6):

$$P_{mi} = \frac{1}{1 + \mathrm{e}^{(-a*(\mathrm{CLi}-\mathrm{b})}}$$

Where a = 0.0908±0.0168 (95% CI) and b = 96.884±1.951 (95% CI).

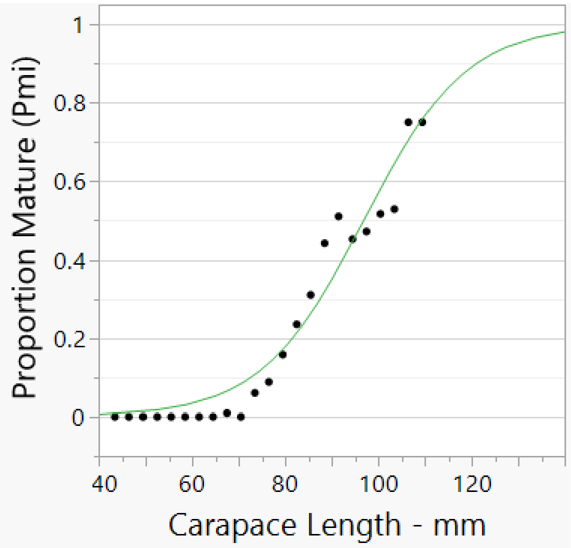

Figure 6. Maturity ogive for lobsters captured in spring in several fishing grounds of the Limfjorden (3 mm classes).

Fecundity (F_i, egg production) was calculated using the power fit model to carapace length (CL_i) from Agnalt (2007) for the number of eggs produced by female lobsters of a given size in South-east Norway:

$$F_i = 0.0045\ CL_i^{3.22}$$

Where F_i is the number of eggs produced and CL_i is carapace length in size class *i*. A power fit model is considered more appropriate than linear models to explain the relationship between body size and egg mass or number (ICES, 2003).

Three indices of spawning potential (ISP) were calculated, which contrary to RRP, also reflect the abundance of mature females: 1) An index of total spawning potential (ISP_t) estimating total egg production using the absolute number of lobsters in size class *i* as N_i was calculated for the MPA and adjacent non-MPA where absolute female population estimates were available. 2) An index of relative spawning potential (ISP_r) indicating relative total egg production, like the ones by Morgan (1982) and Goñi et al. (2003), was calculated as:

$$ISP_r = F * Pm * CPUE$$

Where, F is the fecundity of the population at mean female size, P_m is the proportion of functionally mature females at mean female size and CPUE is catch per unit of effort (lobsters per pot per day), an indicator of lobster density. 3). Additionally, an index of spawning potential per area (ISP_a) reflects lobster population size by multiplying ISP_r by the area (km^2) occupied by the three populations (MPA = 0.298 km^2, non-MPA = 0.290 km^2 and control fished = 2.3 km^2).

2.3.9 Data analysis

Normality of data was tested using Anderson-Darling's test and normal probability plots, while heterogeneity of variance was determined using Levene's test. Standard or paired t-tests were used to determine differences in normal data with equal variance, while the non- Kruskal-Wallis test and Dunn's pairwise test with Bonferroni correction, were used to determine differences in non-normal data. Spearman's rank correlation was used to determine relationships between non-normal distributed variables. All assume a significance probability of $\alpha \leq 0.05$.

2.4 Lobster population estimates

2.4.1 Population estimates

A total of 420 lobsters were marked in survey A, with 10% or 42 tagged lobsters being recaptured in survey B, which also fished a total of 420 lobsters (Table 3). The size of the lobster population was estimated separately for the MPA and non-MPA (Figure 3; Table 3). Even though the fished adjacent non-MPA is a narrow band surrounding the MPA and thus assumptions of the CMR method may not be fully met, recaptured lobsters indicate limited migration between the MPA and non-MPA.

Total population estimates were 2,827±808 (95% CI) lobsters in the MPA and at 1,326±799 (95% CI) lobsters in the non-MPA, or 47% of MPA (Table 3). The adult/harvestable fraction was estimated at 1,600±458 (95% CI) lobsters in the MPA and 459±277 (95% CI) lobsters in the non-MPA, or 29% of MPA (Table 3).

Female lobster population was estimated at 964±276 (95% CI) in the MPA and 47% lower in the non-MPA at 452±272 (95% CI) (Table 3). Male lobster population was estimated at 1,862±532 (95% CI) in the MPA and 47% lower in the non-MPA at 873±526 (95% CI) (Table 3).

Lobster density was estimated at 9.5±2.7 per 1,000 m^2 (95% CI) in the MPA, 2.1 times the density in the adjacent non-MPA of 4.6±1.8 per 1,000 m^2 (95% CI) (Table 3). Harvestable density was 5.4±1.5 per 1,000 m^2 (95% CI) in the MPA, 3.4 times higher than in the adjacent non-MPA at 1.6±1.0 per 1,000 m^2 (95% CI) (Table 3).

Table 3. Lobster population size (N) estimated for the MPA and non-MPA (Figure 2) using the Chapman adaptation of the Lincoln-Petersen method for single mark-recapture: N is the estimated number of animals in the population, n is number of animals marked in first visit, K is the number of animals captured in the second visit and k is the number of recaptured marked animals. F are estimates for female lobsters and M are estimates for male lobsters, using the proportion of sexes observed in both surveys (34.1% females, N = 840). Shown is total population and adult/harvestable fraction, i.e. larger than the minimum landing size of 87 mm carapace length. Densities (lobsters/1 000 m2) were calculated using the surface area of each site (Section 2). Error is 95% confidence interval.

	MPA			Non-MPA		
	Total	F	M	Total	F	M
n	316			104		
K	320			100		
k	35			7		
% Recapture	11.1			6.7		
Population	2,826	964	1,862	1,325	452	873
	±808	±276	±532	±799	±272	±526
Adult/Harvestable	1 600			459		
Fraction	±458			±277		
Density	9.5	3.2	6.3	4.6	1.6	3.0
	±2.7	±0.9	±1.8	±2.8	±0.9	±1.8
Adult/Harvestable	5.4			1.6		
Density	±1.5			±1.0		

2.4.2 Discussion

Not all the assumptions of the CMR method were fully met, namely the assumption of closed system. Even if the home range of European lobsters is smaller or similar in size to the surveyed area (0.59 km^2), 0.04 to 0.64 km^2 (Moland et al, 2011a; Wiig et al., 2014), European lobsters are known to cover significant distances (several hundred meters) in a few days to weeks (e.g. Moland et al., 2011b, unpublished Hummerfiskeri EMFF project). The recapture of tagged lobsters during the study in areas other than their tagging area provides evidence of migration, and thus that the closed system assumption was not met. Captures by fishermen provided further evidence of movement at larger scales. However, the magnitude of migration between areas during the CMR study was small: one lobster (2.9% of recaptures) emigrating from the MPA and 3 lobsters (8.6% of recaptures) immigrating to the MPA between marking and recapture surveys.

Even if net migration is not zero, if the probability of migration is the same for tagged and non-tagged lobsters, changing K and k by the same proportion, the impact on population estimates of the observed migration between marking and recapture is small. Only a 0.12% decrease in population estimates for the observed 5.7% net immigration to MPA or a 2.6% increase in population estimates for the 8.6% net emigration to non-MPA observed between surveys. Therefore, deviations from the CMR closed system assumption have only a limited small impact on lobster population estimates.

2.4.3 MPA vs non-MPA lobster populations

Positive effects on lobster abundance from MPA fishing protection and/or reef habitat were clearly observed in total lobster density and particularly in adult/harvestable density in the MPA that were respectively over twice and three times those in the non-MPA, a significant difference considering the 95% confidence intervals (Table 3). Even after the short time of 3 years since the LSR-MPA was established in 2018, a significant positive effect from MPA fishing protection can be expected as the one observed. Significant increases of similar magnitude in European lobster abundance (ca. 2.5 times) were observed after only 4 years from the establishment of MPAs in the UK and Norway, albeit larger MPAs ranging in area from 0.5 km^2 to 4 km^2 (Hoskins et al., 2011; Moland et al 2013a; Kleiven et al., 2019). Increases in lobster abundance due to MPA effects can result from increased survival (i.e. reduced fishing mortality), higher retention and limited export of lobsters (Hoskins et al., 2011; Moland et al., 2011a,b; Huserbråten et al., 2013; Kleiven et al. 2019; Fernández-Chacón et al., 2021), but also from large lobsters migrating into the MPA (Thorbjørsen et al., 2018) and likely the provision of rare valuable habitat (i.e. the reefs; Jensen et al., 2000).

2.5 Lobster mobility and spill-over

2.5.1 Survey recaptures of marked lobsters

47 lobsters were recaptured during the two CMR surveys (includes lobsters recaptured in the same survey when marked): 39 marked in the MPA (12 of which in reefs) and 8 marked in the adjacent non-MPA (Figure 7; Table 4). Seven recaptured lobsters migrated from their marking area (14.9%): two lobsters marked in MPA were recaptured in non-MPA (5.1%), while 5 lobsters marked in non-MPA where recaptured in the MPA (62.5%), resulting in a net immigration of 3 lobsters into the MPA (6.4%). The median movement distance was 64 m and ranged between 2 and 476 m (Figure 8; Table 4).

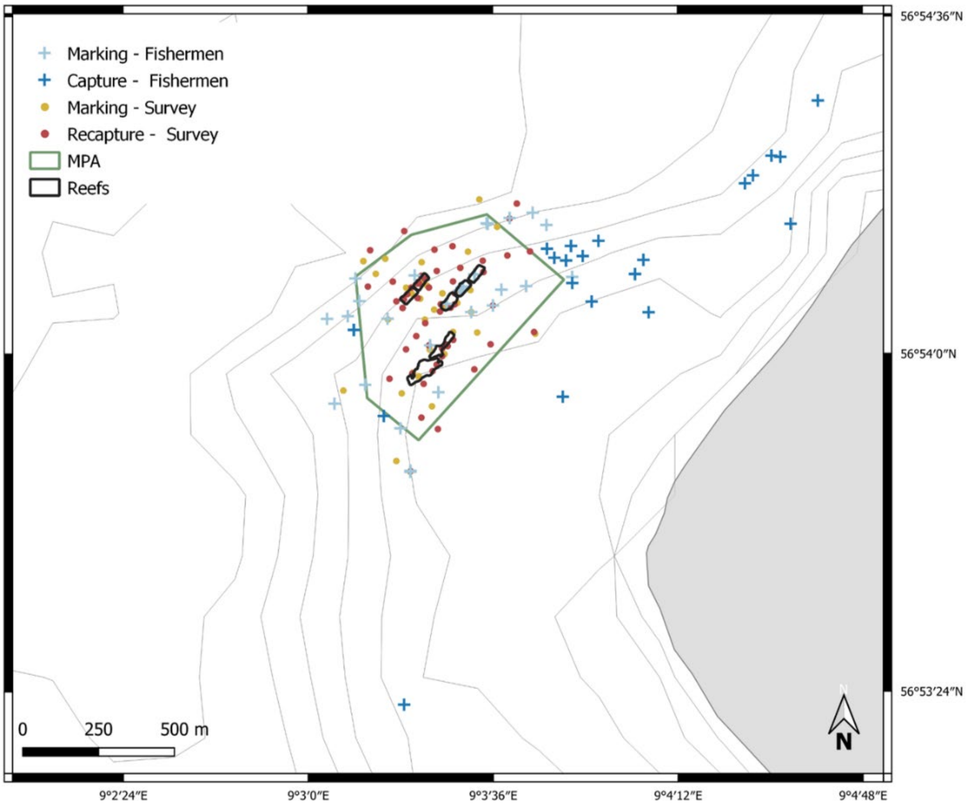

Figure 7. Marking and recapture locations of lobsters during the CMR surveys (yellow and red dots) and by fishermen (light and dark blue crosses).

2.5.2 Fishermen captures of marked lobsters

A total of 30 lobsters were captured by fishermen outside the MPA after the CMR surveys: 18 marked in MPA (7 of which in reefs) and 12 marked in adjacent non-MPA (Figure 7; Table 4). Eighteen captured lobsters migrated from the MPA to non-MPA, which correspond to 3.0% of all marked lobsters in the MPA (N = 595). Seven lobsters were marked in reefs, which correspond to 3.7% of all marked lobsters in reefs (N = 190). Fifteen were larger than minimum landing size (MLS) or 4.3% of all marked lobsters larger than MLS, weighing a total of 19.4 kg (N = 349). No migration from non-MPA to MPA can be assessed from fishermen recaptures. The median movement distance was 594 m and ranged between 49 and 1 586 m (Figure 8; Table 4). If tagged lobsters were caught by other fishermen and not reported, the implication would be that the estimated spillover is a minimum estimate.

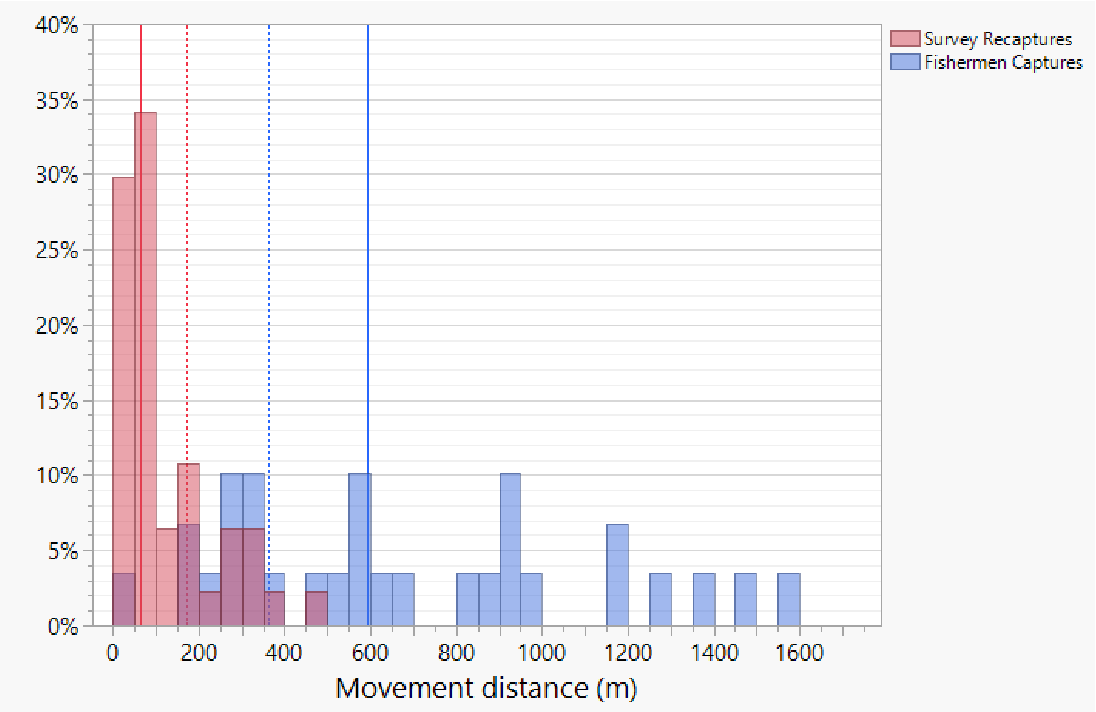

Figure 8. Movement distance (m) of lobsters between marking and recapture in the CMR surveys (red) and post-survey by fishermen (blue). Vertical lines mark median distances travelled by lobsters marked in the MPA (solid) or non-MPA (dashed).

Table 4. Number of recaptured lobsters, number of lobsters that migrated to a different area, median, minimum (Min) and maximum (Max) movement distance for survey recaptures and post-survey fishermen captures relative to their marking location: entire surveys, on reefs, in the MPA and in the fished adjacent non-MPA (Figure 3).

Marking Area		**Migrated**	**Distance (m)**		
	N	N	Median	Min	Max
Survey					
All	47	7	64	2	476
Reef	12		53	9	306
MPA	39	2	61	4	476
Non-MPA	8	5	172	2	343
Fishermen					
All	30		581	49	1 586
Reef	7	7	579	251	1 391
MPA	18	18	594	251	1 586
Non-MPA	12		361	49	1 477

2.5.3 Movement

Movement distance was not significantly different between lobsters marked in the MPA or non-MPA and recaptured in the CMR surveys (*log10* transformed, *t*-test, $t = 0.38$, $p = 0.80$) or in fishermen captures (*t*-test, $t = 1.22$, $p = 0.24$). Movement distance was not correlated with duration between marking and recapture either in the CMR surveys (*log10* transformed, $r = -0.22$, $p = 0.14$, $N = 47$) or fishermen captures ($r = 0.24$, $p = 0.21$, $n = 30$).

Lobsters did not move to different water depths as no significant difference between water depth of marking and recapture was observed in both the CMR surveys (paired t-test, *t* = 1.94, p = 0.058) and fishermen recaptures (paired t-test, *t* = 1.14, p = 0.263).

Polar diagrams of lobster movement indicate that movement during CMR surveys varied across multiple directions with no clear alignment, while movement in fishermen recaptures was aligned along a SW-NE axis, at 240 – 60 degrees, roughly parallel to bathymetry (Figures 8 and 9). However, location of fishing gear may favour specific depths and locations and thus likely bias the observed distance and direction of captures.

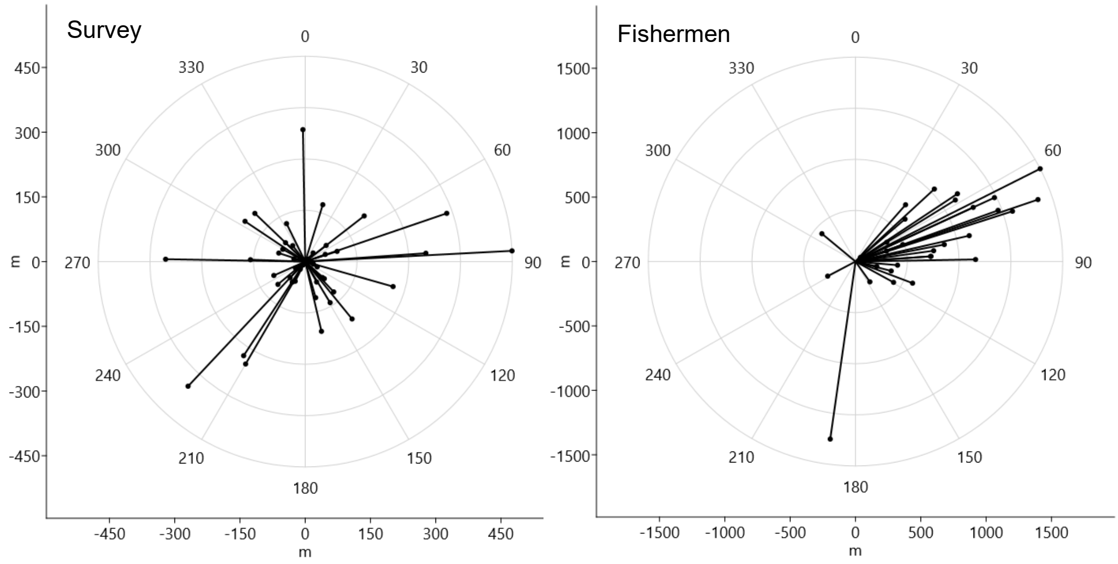

Figure 9. Polar plots of lobster movement distance and cardinal direction between marking and recapture in the CMR surveys (left) and in fishermen recaptures (right).

2.5.4 Spillover from LSR-MPA to adjacent fishing grounds

We observed lobster movement into and out of the LSR-MPA, from distances of several hundred meters to over 1,000 m within a few days to a few weeks, indicating frequent and rapid migration between the LSR-MPA and surrounding areas. This agrees with the scale of short-term movement (days to weeks) of 100 m to over 1,000 m (e.g. Skerrit et al., 2015; Thorbjørsen et al., 2018), and high site fidelity with home ranges (95% utilization) from <1.000 m^2 to 0.04 km^2 described for European lobsters in the UK and Norway (e.g. Skerrit et al., 2015; Moland et al., 2011a,b). European lobsters, however, may be facultatively territorial or migratory according to size-related habitat requirements, with a fraction of lobsters much less mobile while others can move over much larger distances (Hoskins et al., 2011). At longer scales, European lobsters can move and migrate up to tens of km in a year to several years (e.g. Jensen et al., 2000; Smith et al., 2001; Huserbråten et al., 2013). However, the Limfjorden may restrict large scale movement as it constitutes a somewhat unique shallow (mean depth 4.5m) and well enclosed habitat, which contrast with common open and deeper coastal European lobster habitats elsewhere in Europe.

The export rate of lobsters marked in the MPA and captured by fishermen outside the MPA was 3.0%, lower than the 4.7% reported by Huserbråten et al. (2013) for Norwegian MPAs. However, Huserbråten et al. (2013) reported from larger MPAs than the LSR-MPA (0.3 km^2 relative to 0.5-1 km^2) and over a longer period of 1 to 2 years than the present study. Larger areas should result in lower export rates than smaller MPAs, if the size of latter is smaller or close to lobster's home range size, while a longer period should result in higher export rates than shorter times as it accumulates captures of tagged lobsters over a longer time.

Using monthly lobster landings in the Limfjorden as a proxy for activity and probability of capture in each month, potential capture of MPA lobsters in other months and an annual export rate from the MPA can be estimated from September landings, the month with measured fishermen captures. Using this approach, the 3.0% capture rate in September 2021 (landings 7 763 kg), correspond to an annual export rate of 7.9% (Table 5), 160% the export rate reported by Huserbråten et al. (2013) for MPAs in the Norwegian Skagerrak. Both our observations and Huserbråten et al. (2013) report captures from fishermen and do not constitute the net export of immigration and emigration to and from the MPAs.

Table 5. Estimation of monthly and annual export rates from the LSR-MPA based on measured fishermen capture rate outside the MPA of 3.0% in September 2021. Monthly lobster landings in the Limfjorden in 2021 were used as a proxy for activity and probability of capture in each month relative to September.

Month	2021 Landings (kg)	Export rate (%)
January	25	0.01
February	4	0.00
March	32	0.01
April	497	0.19
May	1,423	0.55
June	3,746	1.45
July	229	0.09
August		0.00
September	7,763	3.00
October	3,882	1.50
November	1,850	0.71
December	1,000	0.39
Annual	20,606	**7.90**

Assuming the probability of emigration from the MPA and capture by fishermen to be the same for marked and non-marked lobsters, then 3.0% or 85±24 (95% CI) lobsters from the MPA are estimated to have been caught in surrounding fishing grounds by a single fisherman in September. Of these, 83.3% or 71±20 (95% CI) lobsters would be larger than MLS and thus could be landed, with a weight of 91.9±25.9 (95% CI) kg estimated from the mean weight of lobsters larger than MLS in the MPA. Its value at auction would be of 18,383±5 180 (95% CI) kr. at 200 kr. per kg (value per kg in September 2021 at Hanstholm Fiskeauktion, Fiskeristyrelsen). The estimated annual capture rate of 7.9% results in the capture of 223±62 (95% CI) lobsters from the MPA in 2021, with 186±52 (95% CI) lobsters larger than MLS with a weight of 242.0±67.7

(95% CI) kg and a value at auction of 52,394±14 653 (95% CI) kr. at 216.5 kr. per kg (value per kg in 2021 at Hanstholm Fiskeauktion, Fiskeristyrelsen).

The scale of lobster mobility from the MPA to surrounding non-MPA areas and the magnitude of fishermen captures of lobsters marked in the MPA and in reefs, therefore provides strong evidence of significant spill-over effects from the MPA to nearby lobster fishing grounds.

2.6 Catches and spatial distribution of lobsters

2.6.1 CPUE in the MPA and non-MPA

Catch per unit effort (CPUE, lobsters per pot per day), an indirect indicator of lobster abundance, was determined for stations classified as being on reefs (R), next to reefs (NR), off-reefs (OR) in the MPA, and in the adjacent non-MPA (Non-MPA) outside the MPA (see Section 2).

CPUE ranged between 0 and 3.04 lobsters/pot/day (Figure 10 and Table 6), and mean CPUE were 0.40±0.04 SE lobsters/pot/day in non-MPA and 0.95±0.04 SE lobsters/pot/day in the MPA. For comparison, in a 2020 survey CPUE in two stations in the MPA and non-MPA were 0.52 and 0.05 lobsters/pot/day, or about 2 and 8 times lower than mean CPUE observed in this study (unpublished, Hummerfiskeri project, EMFF).

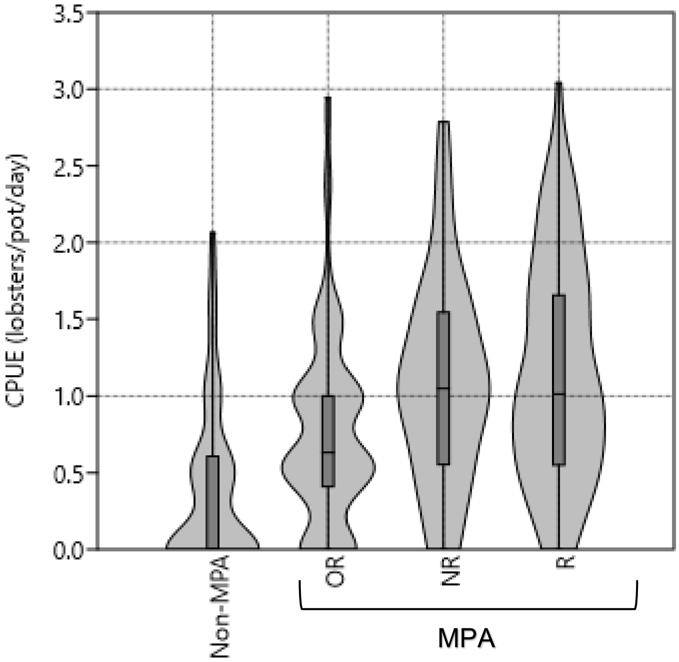

Figure 10. Violin and box plots of CPUE (lobsters/pot/day) in the MPA on reefs (R), next to reef (NR) and off-reef (OR) and in non-MPA with median, quartiles and outliers. Violin plot shows kernel density (continuous histogram) ranging from minimum to maximum values. CPUE distribution is non-normal and variance unequal (Anderson-Darling test, A2 = 13.36, p = 0.0001; and Levene test, F = 4.42, p = 0.0045).

CPUE in the MPA was significantly higher than in non-MPA (Kruskal-Wallis: *H* = 80.62, *p*<0.0001) and all areas of the MPA, i.e. R, NR and OR, showed significantly higher CPUE than non-MPA (Dunn: p<0.0001).

Within the MPA (Figure 10 and Table 6), CPUE was not different between R and NR (Kruskal-Wallis: *H* = 0.15, *p* = 0.697), but both were significantly higher than in OR areas (Dunn: p = 0.0005 and p = 0.0012, respectively). No significant difference was observed in CPUE between the three reef units (Dunn: p>0.05), which were made from different stones and with different layouts (Section 2). Therefore, both R and NR areas are interpreted to represent reef habitat, where the CPUE in the immediate vicinity to the reefs (i.e. < 60m) reflects a common lobster population associated with the reefs.

Table 6. Mean CPUE (lobsters/pot/day) for all lobsters, female and male lobsters in the MPA and in the non-MPA, as well as in three areas in the MPA: reef (R), next to reef (NR) and off-reef (OR). Significant differences from non-parametric Kruskal-Wallis and Dunn tests (p<0.05).+ number of stations and * number of lobsters.

			All			Female			Male	
Area	Significant difference	N^+	CPUE	SE	N*	CPUE	SE	N*	CPUE	SE
R	OR, Non-MPA	72	1.09	0.08	63	0.41	0.05	112	0.70	0.06
NR	OR, Non-MPA	58	1.14	0.09	44	0.33	0.05	109	0.80	0.08
OR	R, NR, Non-MPA	113	0.76	0.06	74	0.27	0.03	125	0.48	0.05
MPA	Non-MPA	243	0.95	0.04	181	0.33	0.02	346	0.62	0.04
Non-MPA	MPA, R, NR, OR	188	0.40	0.04	65	0.15	0.02	110	0.25	0.03

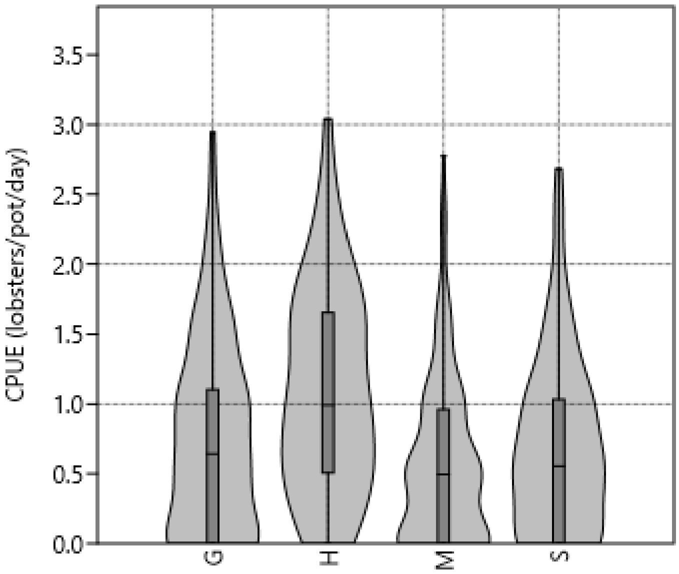

Figure 11. Violin and box plots of CPUE (lobsters/pot/day) by substrate: Hard-boulders (H), grave (G), mixed gravel-sand (M) and sand (S). Violin plot shows kernel density ("continuous histogram") ranging from minimum to maximum values. CPUE distribution is non-normal and variance unequal (Anderson-Darling test, A2 = 13.36, p = 0.0001; and Levene test, F = 4.42, p = 0.0045).

2.6.2 CPUE of male and female lobsters

Catches were different according to sex with females representing only 54% of male catches, and CPUE was significantly different for female and male lobsters (Table 6; Kruskal-Wallis: $H = 34.92$, $p<0.0001$).

CPUE for both female and male lobsters was different between the MPA and non-MPA (Kruskal-Wallis: Females: $H = 32.64$, $p < 0.0001$; Males: $H = 57.57$, $p<0.0001$), being higher in the MPA than in the non-MPA (Table 6).

Within the MPA, female CPUE was similar in the three areas R, NR and OR (Kruskal-Wallis: $H = 3.489$, $p>0.05$), while male CPUE was similar in R and NR (Dunn: $p<0.0085$), CPUE in both were significantly higher than in OR (Dunn: $p = 0.0083$ and $p = 0.0005$).

2.6.3 Spatial variation of CPUE and environmental variables

Of the environmental variables, CPUE was unrelated to depth (Spearman rank correlation, $\rho = -0.04$, $p > 0.05$).

CPUE was significantly different between substrates (Figure 11; Kruskal-Wallis: $H = 22.82$, $p<0.0001$), being higher in hard-boulders (H) than in mixed (M) and sand (S) substrates, as well as being higher in gravel (G) than in mixed substrate (Dunn: all $p<0.05$). CPUE in gravel was not different from hard-boulders and sand (Dunn: all $p>0.05$).

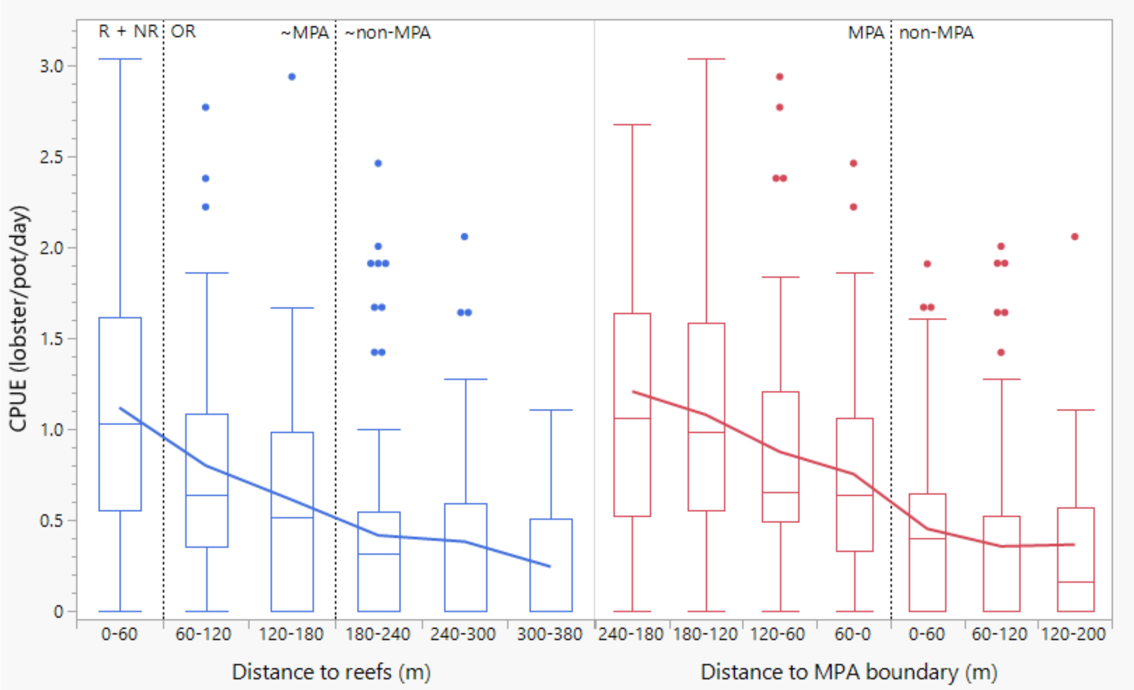

Figure 12. Box plots of CPUE (lobsters/pot/day) per 60 m classes relative to distance to closest reef (left) and distance to MPA boundary (right) showing medians, quartiles and outliers. Vertical dotted lines mark separation between different habitats (reef habitat: R+NR and off-reef habitat: OR) and protection (no-take MPA and fished non-MPA). Lines connect mean CPUE.

CPUE was significantly negatively correlated with both distance to closest reef and distance to the MPA boundary (Spearman rank correlation, respectively $\rho = -0.47$ and -0.45, both *p*<0.0001). Both female and male lobsters CPUE were significantly negatively correlated with both distance to closest reef and distance to the MPA boundary (Spearman rank correlation, Females: respectively ρ = -0.27 and -0.27, both *p*<0.001; and Males: respectively ρ = -0.41 and -0.40, both *p*<0.001). However, distance to closest reef and distance to the MPA boundary significantly co-vary as the reefs occupy a central location inside the MPA (Spearman rank correlation, ρ = -0.95, *p*<0.0001).

Once categorized into 60 m wide classes of distance to closest reef and distance to MPA boundary, CPUE showed significant differences (Kruskal-Wallis: *H* = 97.77, *p*<0.0001 and *H* = 94.14, *p*<0.0001, respectively), decreasing with distance from reefs and to MPA boundary (Figure 12). CPUE was significantly higher in the 120 m closest to the reefs than from 180m away from the reefs, roughly corresponding to the MPA boundary (Dunn: all *p*<0.01). While CPUE was not significantly different with distance inside the MPA (Dunn: all *p*>0.05), all distance classes inside the MPA where significantly higher than more distant classes in non-MPA (Dunn: all *p*<0.05). Distances to reefs and to MPA boundary were strongly correlated (Spearman rank correlation, ρ = 0.95, *p*<0.0001) since the reefs occupy a central location of the MPA. Thus, its relationships with CPUE reflecting reef habitat effects and MPA protection effects on lobster abundance cannot be differentiated.

2.6.4 Modelling lobster abundance distribution

General linear mixed models (GLMM) allow to model lobster abundance distribution as a function of multiple variables. However, GLMM modelling results showed distance to reef and depth had the lowest AICc values and were the only explanatory variables ones with statistical significance (Table 7).

Table 7. General linear mixed model of lobster abundance with depth and distance to reefs as explanatory variables. Lobster abundance was best described by a negative binomial probability distribution (nbinom1). Parameter estimates expressed on the log-link function scale and inverse-link transformed under Effect on abundance factor. * indicates statistical significance (p<0.05). Effect of explanatory variables on abundance was scaled with Effect on abundance factor. Dispersion factor of the model is 0.096.

GLMM Model	Variable	Parameter estimate	Parameter probability	Effect on abundance
	Intercept	0.478	<0.0001*	~0
	Depth	0.0547	0.014*	1.056
	Distance	-0.0031536	<0.0001*	0.997

Model validation showed a bias toward neither overestimation nor underestimation of abundance, but significant error with both median RMSE and MAE larger than mean CPUE 1.38± 0.058 (SE, here only expressed as lobster/pot/48h), and low Rsq, indicating the model fits well the data, but is poor at predicting new lobster abundances (Figure 13). Since lobster abundance/CPUE was different between sexes, it is possible the GLMM model could be improved by including sex as an explanatory variable or modelling sexes separately. It is also possible that the soak time as an offset of abundance is insufficient to correct for gear saturation effects at 5 and 6 soak days and these should be excluded from analysis. However, since the GLMM does not provide additional explanation of lobster distribution than univariate analysis, further modelling was not performed.

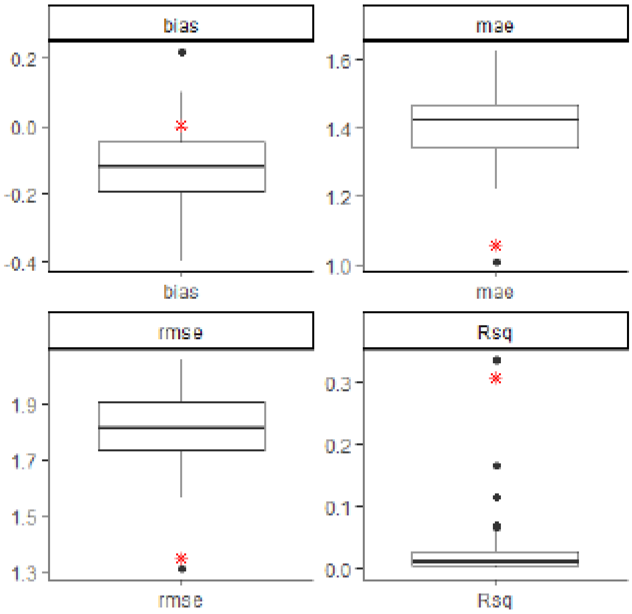

Figure 13. Boxplots of bias, MAE, RMSE, and Rsq, derived from 50 iterations where 90% of the whole data set was randomly sub-sampled and used to train the model, and the remaining 10% data used for validation. The red star (*) indicates model predictions from the whole data set.

2.6.5 Discussion

The observed skewness of catch sex ratio to males (ca. 2 males per 1 female) may either represent a real skewness of the lobster population sex ratio toward males or sex-related differences in catchability where spawning, moulting and egg extrusion from late spring to early autumn reduce female catchability (ICES, 2003, Wahle, 2013). However, catch report data from the size control fished site in Ejerslev Røn (Sections 6 and 7), observed a similar skewness of catch sex ratio toward males both in spring and autumn of 2020 and 2021 (unpublished, Hummerfiskeri project, EMFF). In contrast, balanced catches sex ratios were observed in other fishing grounds further south in the Limfjorden (Fur, Kås and Venø) both in spring and autumn 2021 (unpublished, Hummerfiskeri project, EMFF). Therefore, seasonal differences in female catchability seem an unlikely explanation for the observed catch sex ratio in the LSR-MPA, which possibly reflect a real skewness in lobster population toward male lobsters.

The observed CPUE variation, a proxy for lobster abundance, provides evidence of positive effects from the artificial reef habitat and/or MPA protection from fishing on the lobster population. Reef habitat (including next to reef areas) had higher lobster abundance than off-reef areas, while the MPA, as well as all its three areas (R, NR and OR) had higher lobster abundance than the adjacent fished non-MPA. Even off-reef areas in the MPA, albeit similar in depth, off-reef habitat and near to non-MPA, had higher lobster abundance than non-MPA. Lobsters CPUE in the MPA (0.95±0.04 lobsters/pot/day) and even the non-MPA (0.40±0.04 lobsters/pot/day) were significantly higher than lobster CPUE (0.11±0.02 lobsters/pot/day) observed across the entire

Limfjorden in the summer 2020 in similar habitats (hard, gravel, mixed or sandy; unpublished, Hummerfiskeri project, EMFF).

However, reef habitat effects and MPA protection effects on lobster abundance cannot be differentiated from each other as reefs have a central location in the MPA, where the protection effect from the MPA is expected to be highest (e.g. Kleiven et al., 2019). Like Kleiven et al. (2019), lobster abundance significantly decreased with distance to the MPA boundary and with distance to reefs.

Reef and next to reef areas are interpreted to both represent reef habitat, sharing a common abundant lobster meta-population associated with reef habitat. The known scale of short-term lobster movement and home range (e.g. Skerrit et al., 2015; Moland et al., 2011a, 2011b), and similar high CPUE and size (see section 6) in both areas suggest a direct influence of the physical artificial reef structures on its immediate vicinity (i.e. <60m) next to reef areas. In addition, a positive effect of the physical reef structures on lobster abundance is supported by higher CPUE on hard-boulder substrate (except than on gravel), which occurs almost exclusively (93.5%) on the reef structures while the other three substrates occur across the MPA and non-MPA (Figure 5). Therefore, reef structures provide a rare habitat in the Limfjorden of large boulders with high refuge density, which likely attracts and retains lobsters.

Regarding the type of stone (larger and angular Norwegian granite in the western and central reefs and smaller, rounded fieldstones in the eastern reefs) and type of structure (high profile, compact well-defined structure in the western and central reefs and low-lying and disperse structure in the eastern reefs) used on reefs, no difference was observed in lobster abundance between three reef groups. However, the three reef groups are close enough to allow the movement of lobsters between them (e.g. Skerrit et al., 2015; Moland et al., 2011a) and thus to affect each other's lobster population.

Effects from reef habitat and/or MPA protection were not the same for female or male lobsters, even though both sexes were more abundant in the MPA than outside in the fished non-MPA. Within the MPA, only male lobster abundance was significantly higher in reef than off-reef habitats, while female lobster abundance was similar in reef and off-reef habitats. MPAs can have a rapid and long-lasting positive effect on lobster abundance and size (Hoskins et al., 2011; Moland et al., 2013a,b), reducing sex specific and size specific mortality imposed by the protection of ovigerous females and minimum landing size (Moland et al 2013a,b; Fernandez-Chacon et al., 2020, 2021). It is thus plausible that male lobsters benefited more than female lobsters from MPA protection, particularly large males (Fernandez-Chacon et al., 2021), leading to larger abundance at the centre of the MPA and/or reefs. Alternatively, male lobsters may also preferentially occupy reefs, outcompeting female lobsters for rare and valued reef habitat.

2.7 Lobster size in the Livø artificial reefs and no-take MPA relative to control areas

2.7.1 Lobster size in the MPA

In the MPA, male lobsters were larger than female lobsters (Kruskal-Wallis: *H* = 49.31, $p<0.0001$) in all three areas, reefs (R), next to reefs (NR) and off-reefs (OR) (Kruskal-Wallis: all,

p<0.002), with a higher proportion >99 mm carapace length (CL), while females were more abundant at CL<97mm (Figure 14).

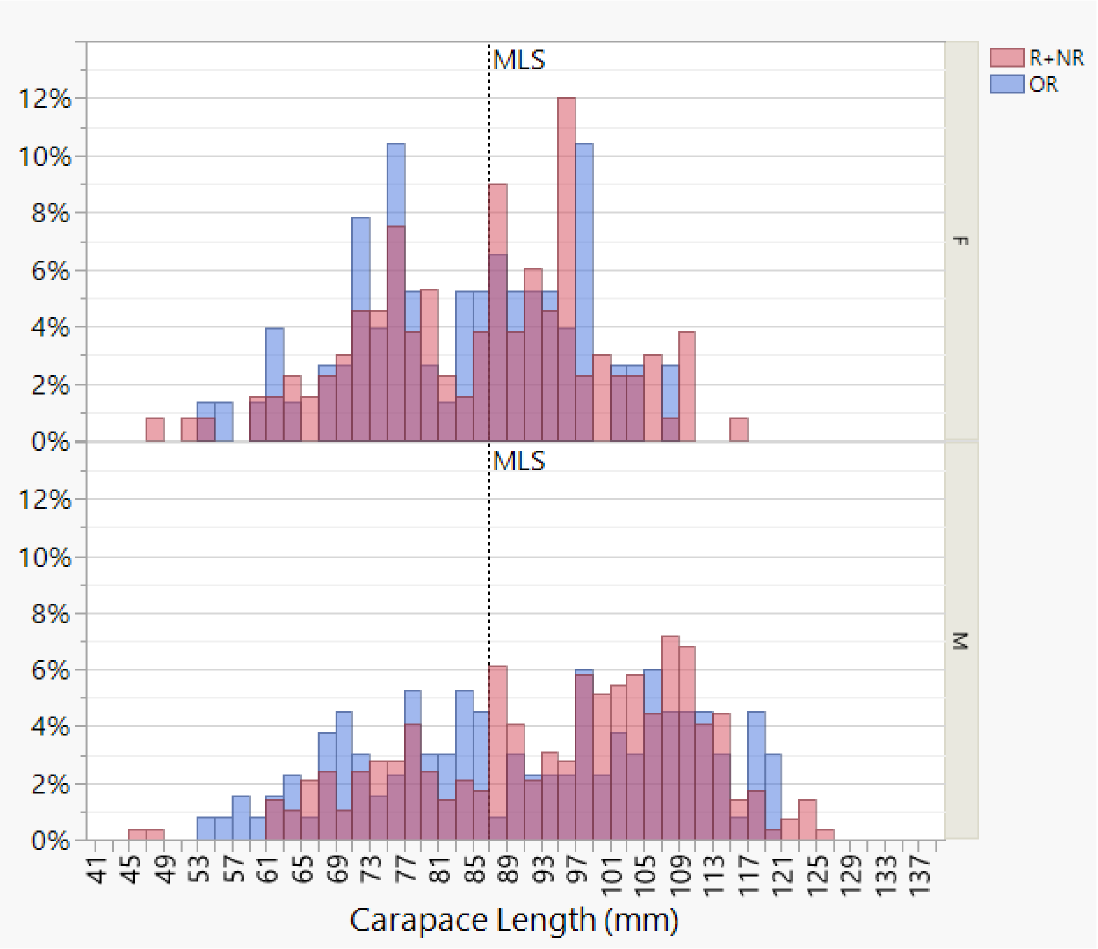

Figure 14. Histograms of lobster carapace length for lobsters captured in the MPA in reefs and next to reefs (red, R+NR) and off-reefs (blue, OR) for both females (F; N = 134 and 77) and males (M; N = 296 and 134). Overlap between the 2 distributions is shown in dark red. Dashed black line is minimum landing size of 87 mm CL (MLS).

Size of female or male lobsters was not significantly different in R, NR and OR areas (Kruskal-Wallis: Females, H = 2.03, p = 0.36 and males, H = 3.51, p = 0.17; Figure 14; Table 8). Mean CL of female lobsters was 84.9±1.55 mm in R, 87.0±1.91 mm in NR and of 83.5±1.46 mm in OR, while mean CL of male lobsters was 95.3±1.20 mm in R, 94.8±1.43 mm in NR and 91.5±1.52 mm in OR (Table 8). Due to similar size in R and NR, and similar CPUE (Section 4), reef and next to reef areas are interpreted has having a shared abundant lobster meta-population associated with reef habitat and are aggregated into one category R+NR (Figure 14).

Table 8. Mean carapace length (mm) of female and male lobsters within the MPA in reef (R), next to reef (NR) and off-reef (OR) areas, and non-MPA areas.

Area	Female			Male		
	N	Mean	SE	N	Mean	SE
R	83	84.9	1.55	164	95.3	1.20
NR	51	87.0	1.91	132	94.8	1.43
OR	77	83.5	1.46	134	91.5	1.52

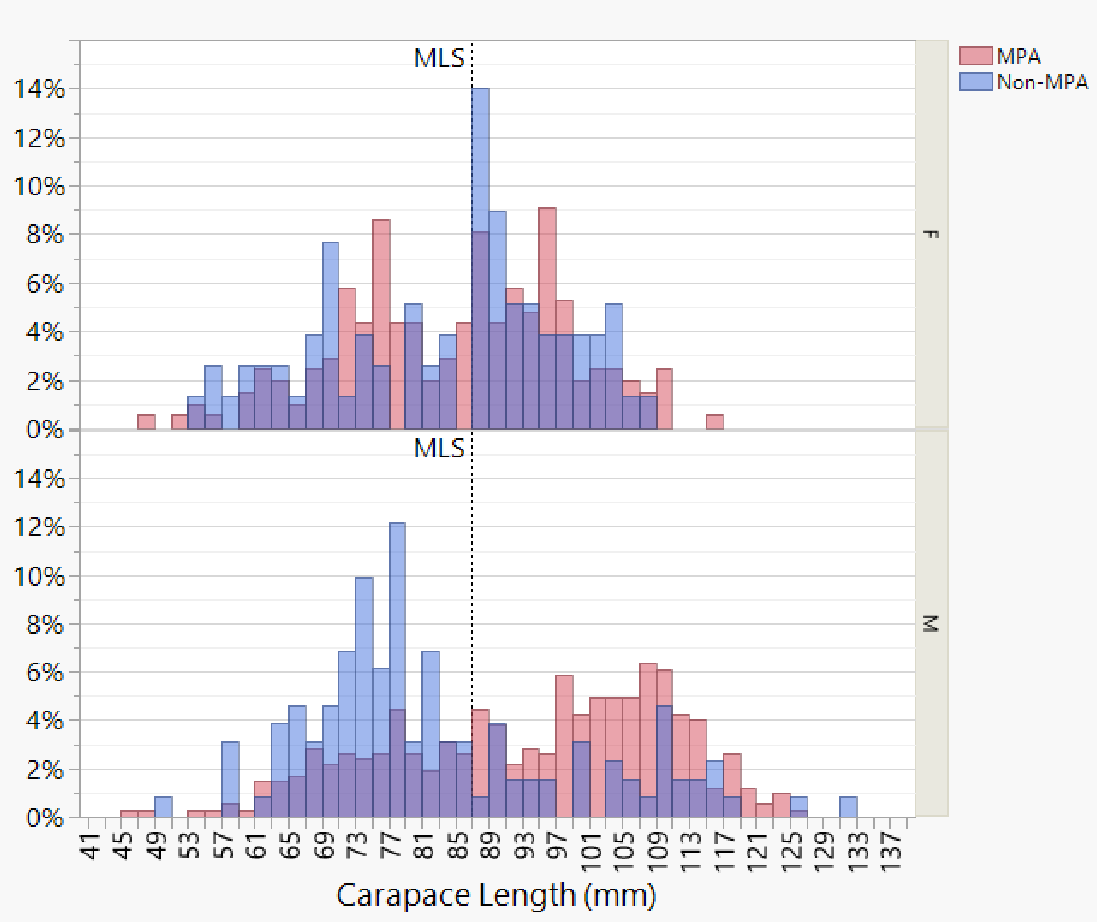

Figure 15. Histograms of female (F) and male (M) lobster carapace length for the MPA (red, female: N = 211, male: N = 430) and for fished adjacent non-MPA (blue, female: N = 79 and male: N = 132). Overlap between the MPA and fished adjacent non-MPA distributions is shown in dark blue. Dashed black line is minimum landing size of 87 mm CL (MLS).

2.7.2 Lobster size in the MPA and adjacent non-MPA

While female lobster size was not significantly different between the MPA and non-MPA (Kruskal-Wallis: $H = 0.23$, $p = 0.63$; Figure 15; Table 9), with all three areas in the MPA similar to non-MPA (Dunn: all $p>0.05$), male lobster size was significantly larger in the MPA than in non-MPA (Kruskal-Wallis: $H = 44.86$, $p<0.0001$; Figure 15; Table 9), in all its three areas (Dunn: all $p \leq 0.0001$).

Table 9. Mean carapace length (mm) of female and male lobsters in the MPA, fished adjacent non-MPA and the control fished site in Ejerslev Røn (Figure 1) for all lobsters and the adult harvestable fraction (larger than MLS).

Area		**Female**			**Male**		
All		N	Mean	SE	N	Mean	SE
	MPA	211	84.9	0.93	430	94.0	0.79
	Non-MPA	79	83.8	1.59	132	82.7	1.41
	Fished	235	77.5	0.73	524	75.3	0.66
Adult							
	MPA	105	96.2	0.64	289	103.7	0.53
	Non-MPA	44	94.4	0.92	38	104.3	1.78
	Fished	36	97.4	1.14	118	96.7	0.67

Female lobster mean CL was 84.9±0.93 mm in MPA and 83.8±1.59 mm in non-MPA (Table 9). Male lobster mean CL was 94.0±0.79 mm in MPA and 82.7±1.41 mm in non-MPA (Table 9). In the non-MPA, the decrease in the frequency of larger male lobsters occurred from a mode at ca. 79 mm, while in the MPA a clear decrease in frequency only occurred from 111 mm (Figure 15).

2.7.3 Lobster size in the MPA and control fished site

The size of both female and male lobsters in the control fished site were significantly smaller than in the MPA and non-MPA (Kruskal-Wallis: females, $H = 40.21$, $p<0.001$ and males, $H = 241.78$, $p<0.0001$; Dunn: all $p\leq 0.0015$; Figure 16). Even the off-reef areas of the MPA and the non-MPA, thus excluding large lobsters associated with reef habitat (R+NR) in the MPA, had larger size than the control fished site (Dunn: females, $p = 0.0017$ and males, $p<0.0001$).

In the control fished site, the decrease in frequency at large sizes was steeper and occurred at smaller sizes than in the MPA (Figure 16). In the control fished site, mean CL was 77.5±0.73 mm for female lobsters and 75.3±0.66 mm for male lobsters, 6.3-7.4mm and 7.4-18.7-mm smaller than in the non-MPA and MPA, respectively (Table 9).

2.7.4 Adult harvestable fraction

Excluding lobsters smaller than MLS, female size was similar between the MPA, non-MPA and control fished site, but male size was significantly different (Kruskal-Wallis: females, $H = 4.57$, $p = 0.102$ and males, $H = 79.75$, $p<0.0001$; Table 9), being higher in the MPA or non-MPA than the control fished site than in (Dunn: both $p\leq 0.0001$; Table 9).

The observed differences between areas in size of the adult fraction according to sex, similar for females and smaller sizes in the control fished site for males, likely reflect differential sex specific mortalities and fishing protection effects from the MPA. The ban on landing ovigerous females reduces female fishing mortality after a certain size once a significant proportion of females is ovigerous, and thus reduces the MPA protection effects. Males on the other hand, benefit from a larger reduction in mortality provided by the MPA as reflected by larger sizes in the MPA and non-MPA relative to the control fished site.

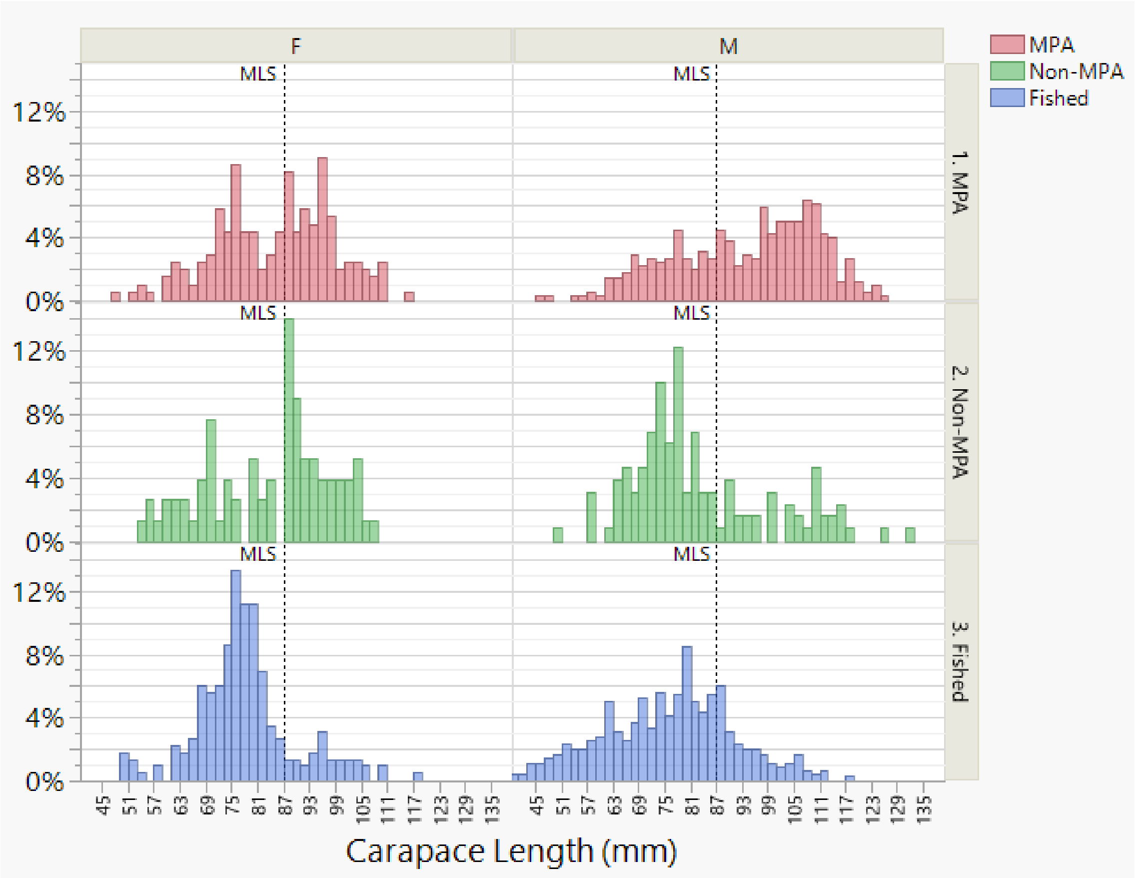

Figure 16. Histograms of carapace length (mm) for female (F) and male (M) lobsters in the MPA (red), fished adjacent non-MPA (green) and control fished site (blue). Dashed black line is minimum landing size of 87 mm CL (MLS).

2.7.5 Length indicators

Two length-based indicators used in fisheries that describe the length frequency distributions of female and male lobsters were determined fitting logistic functions to the cumulative proportion of catches by carapace length (CL): Length at first capture (L_{50}) and length at 95% catch (L_{95}) (Figure 17). L_{50} is the length at which 50% of the population is retained by the fishing gear. L_{95} is the length at which 95% of the population is retained by the fishing gear (L_{95}), an indicator for the presence of the largest length groups.

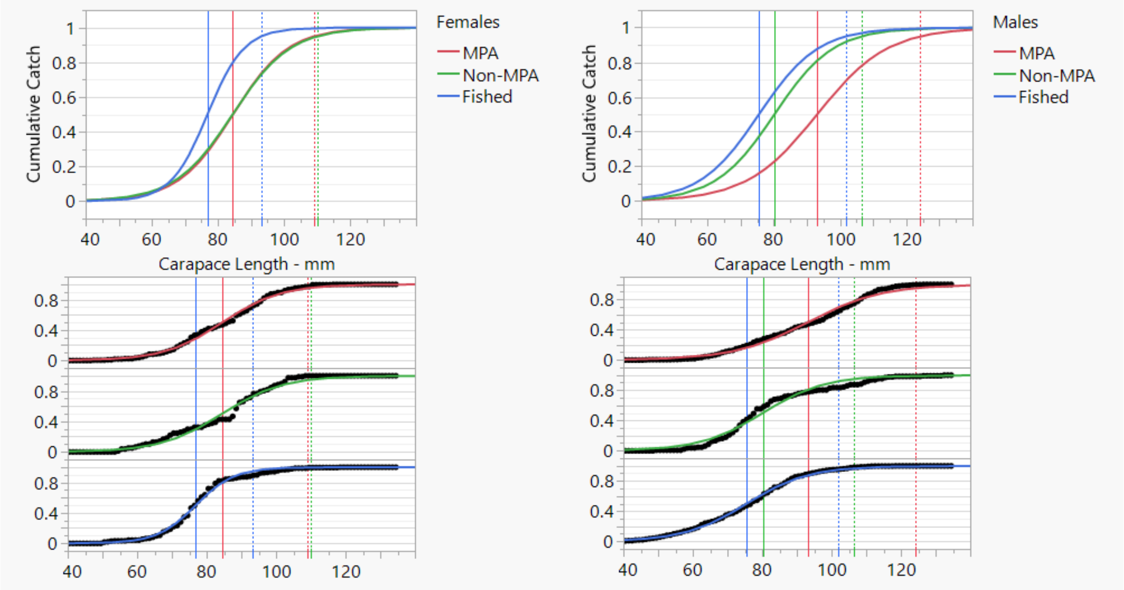

Figure 17. Logistic fits to the cumulative proportion of catches of female and male lobsters by carapace length at MPA (red), fished adjacent non-MPA (green) and the control fished grounds at Ejerslev Røn (blue). Solid lines are L_{50} and dashed lines are L_{95} for the different areas (see text for definitions).

Both L_{50} and L_{95} of female and male lobsters were higher in the MPA and non-MPA than in the control fished site (Figure 17, Table 10). L_{50} and L_{95} of male lobsters decreased across the fishing mortality gradient, being higher in the no-take MPA, intermediate in adjacent fished non-MPA and lowest in the control fished site (Figure 17, Table 10). In contrast, L_{50} and L_{95} of females were similar in the MPA and non-MPA (Figure 17, Table 10).

Table 10. Length at first capture (L_{50}), the length at which 50% of the population is retained by the fishing gear. Length at which 95% of the population is retained by the fishing gear (L_{95}), an indicator for the presence of the largest length groups in the catch. L_{50} and L_{95} were estimated from logistic fits to the cumulative proportion of catches of female and male lobsters per carapace length (mm).

Area	L_{50}	95% CI	L_{95}	95% CI	N
Female lobsters					
MPA	84.8	±0.39	109.2	±1.07	211
Non-MPA	84.1	±0.40	109.8	±1.10	79
Fished	76.8	±0.32	93.2	±0.89	235
Male lobsters					
MPA	94.5	±0.53	124.9	±1.49	430
Non-MPA	80.4	±0.49	106.7	±1.37	132
Fished	75.5	±0.49	101.8	±1.36	524

L_{50} was smaller than the minimum landing size (MLS) of 87 mm in all areas and for both sexes, except for male lobsters in the MPA (Table 10). The difference between L_{50} to MLS for female and male lobsters in the control fished site requires at least 2 moults, possibly 3-4 moults, at 5-

10% growth increment per moult (Wahle et al., 2013 and references therein), while in the MPA female lobsters L_{50} is less than 0.5 moult from increment from MLS and is already larger than MLS for male lobsters (Table 10).

L_{95} confirms that female lobsters in both the MPA and non-MPA reach larger maximum sizes than in the control areas (Table 10), and that male lobsters in the MPA reach larger maximum sizes than in the control and non-MPA fished sites (Table 10).

2.7.6 Discussion

MPAs positively impact lobster size distribution by increasing survival, reversing the negative relationship between survival and body size (Hoskins et al., 2011; Moland et al., 2013a,b; Fernandez-Chacon et al., 2020; 2021). Growth in the short time of 3 years since LSR-MPA implementation can account for a significant portion of the size differences between the MPA and non-MPA with the control fished site, particularly regarding smaller lobsters. For instance, differences in mean sizes and L_{50} between the three areas can be achieved in 3 years assuming 5-10% growth increment per moult (Wahle et al., 2013 and references therein) and the probably of moulting and multiple moulting per year at those sizes (Coleman et al., 2020). However, with the decrease in moult increment and moult frequency with size (Wahle et al., 2013, Coleman et al., 2020, and references therein), the differences in the largest sizes between the three areas, expressed in L_{95} cannot be explained by growth alone. Such differences require large lobsters migrating into and being retained in the MPA as observed in other MPAs in Norway (Thorbjørsen et al., 2018). On the long term (>10 years), MPAs can reduce sex specific mortality, reverse the negative relationship between survival and body size and increase survival, leading to increases in body size and abundance (Moland et al., 2013a,b; Fernandez-Chacon et al., 2020; 2021).

Since fishing regulations impose a sex specific protection of ovigerous females and increase mortality of larger sizes through a minimum landing size, it is possible that male lobsters benefited more than female lobsters from MPA protection and/or the reef habitat, as MPAs lead to a long-term decrease in sex specific mortality (Moland et al 2013a,b). Large male lobsters have been reported to benefit more than smaller male lobsters from MPA protection (Fernandez-Chacon et al., 2021), which also results in larger claws in male lobsters (Sørdalen et al., 2019).

2.8 Lobster reproductive and spawning potential in the Livø MPA relative to fished sites

Relative Reproductive Potential (RRP) reflects the impact of size distribution of the female population and fecundity with size on the potential to produce eggs and does not consider female lobster abundance. Indices of spawning potential (ISP) in addition to size and fecundity, also reflect the female lobster abundance. ISP_t estimates absolute egg production (i.e. number of eggs) using the absolute number of mature females per size obtained in Section 3. ISP_r and ISP_a, use CPUE as a proxy for female lobster abundance applied to a mean female lobster of mean size, mean ovigerous proportion and mean fecundity. Additionally, ISP_a incorporates fishing ground area to scale CPUE with the area occupied by the lobster populations.

2.8.1 Results

MPA and non-MPA had similar RRP (Table 11) of ca. 300,000 as female size was similar in the two areas, (Table 9, Section 6), but RRP in both areas was twice higher than in the control fished site due to the smaller size of females in the latter (Table 11).

Total egg production (ISP_t) was twice as high in the MPA than in the non-MPA (Table 11), 3.1 million eggs and 1.4 million eggs respectively, reflecting the higher lobster density in the MPA (Table 3; Section 3), as female lobster size was similar in both areas. No estimates of ISP_t could be made for the control fished site as an estimate of population size is not available.

ISP_r showed a decrease from MPA to non-MPA to control fished site, reflecting a decrease in female lobster abundance and size with increasing fishing mortality: ISP_r was highest in the MPA, with non-MPA ISP_r at 50% the MPA, and was lowest in the control fished site at 25% the MPA (Table 11). ISP_a on the other hand, was highest in the control fished site followed by the MPA and lowest in the non-MPA (Table 11).

Table 11. Indices of relative reproductive potential (RRP), total spawning potential (ISP_t), and relative spawning potential (ISP_r and ISP_a). Error is 95% confidence intervals.

Area	RRP	ISP_t	ISP_r	ISP_a
MPA	319,139 (±35 012)	3,076,503 (±880,824)	544.3 (445-661)	161.9 (132-197)
Non-MPA	308,224 (±33 953)	1,393,172 (±838,369)	238.7 (168-332)	69.3 (49-96)
Control	158,429 (±24 585)		135.2 (120-152)	311.0 (276-350)

2.8.2 Discussion

Protection and/or habitat effects provided by the LSR-MPA have a significant impact on the reproductive potential of its lobster population, due to increased lobster body size and abundance relative to the fished non-MPA and control sites. Since fecundity in lobsters increases significantly with body size (Tully et al., 2001; Agnalt, 2007; ICES, 2003) – the fecundity of 2 lobsters at MLS of 87 mm equals a single lobster of 108 mm carapace length at ca. 15,800 eggs – size differences between areas have a significant impact on reproductive and spawning potential. While most of the reproductive and spawning potential in the MPA and non-MPA originates from larger lobsters at sizes larger than MLS, 88 and 96% respectively (Figure 18), in the control fished site sizes only 20% originates from lobsters larger than MLS, with most of the reproductive and spawning potential coming from between 71-83 mm CL (Figure 18). Therefore, larger body size in the MPA and non-MPA results in a RRP in both areas that is twice that of the control fished site (Table 11). However, higher lobster density in the MPA result in its absolute egg production (ISP_t) being twice than of adjacent non-MPA even though female lobster size was the same in both areas.

A combination of larger size and higher abundance (CPUE) in the MPA results in relative spawning potential (ISP_r) being twice than in adjacent non-MPA and 4 times that of the control fished site. Since the control fished site area is 7.7 times larger than the MPA and non-MPA (MPA = 0.298 km^2, non-MPA = 0.290 km^2 and control fished = 2.3 km^2), the control fished site lobster population has a higher area spawning potential (ISP_a), which however is only ca. 2 times larger than in the MPA. Even though absolute population size is not known for the control fished site, and thus estimation of absolute egg production is not possible, ISP_a suggests it should be about twice that of the MPA, and thus that egg production in the MPA is equivalent to a fished ground ca. 4 times larger.

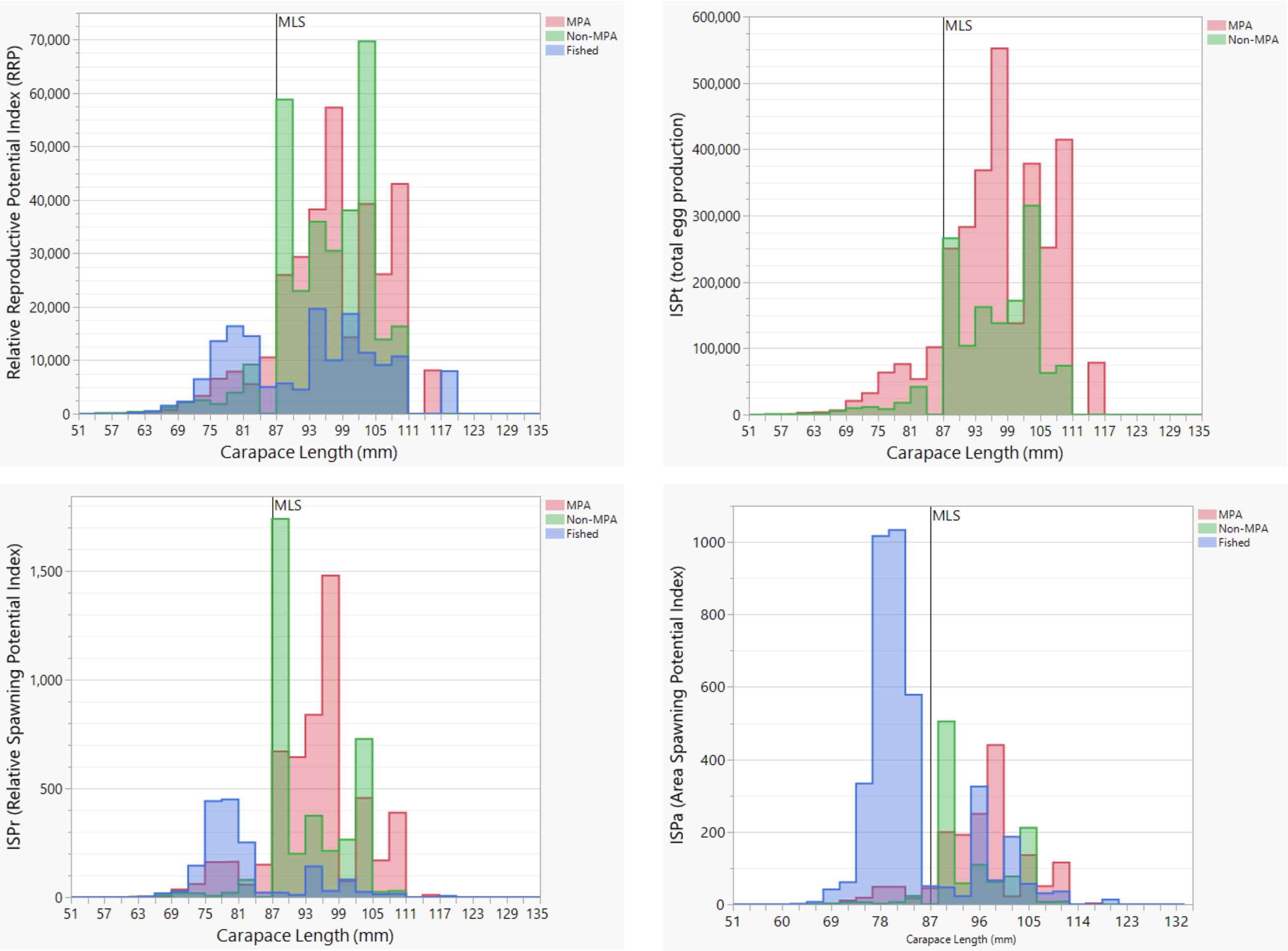

Figure 18. Relative reproductive potential index (RRP, top left), total spawning potential (ISP$_t$, top right), relative spawning potential (ISP$_r$, bottom left) and area spawning potential (ISP$_a$, bottom right) relative to size (carapace length) for the MPA (red), non-MPA (green) and control fished site (blue). Refer to the text and section 2 for a definition of the indices.

2.9 Shore crab distribution

Only opportunistic non-quantitative data of shore crab abundance in the LSR-MPA was collected profiting from the lobster survey, as lobster pots are not fully adequate to survey shore crab populations. Nevertheless, and in spite of non-abundance related factors (e.g. loss of crabs when hauling the pot), it can be expected that crab catch data roughly reflects local abundance during the surveys, but absolute catch data obtained is not comparable to other studies.

2.9.1 Results

Crab catches, CPUE (catch per unit effort as crabs per pot per day) ranged 0 and 10.19 crabs/pot/day, with very low catches of female crabs (Figure 19 and Table 12).

Crab CPUE was significantly lower in the MPA than in the non-MPA (Kruskal-Wallis: $H = 69.81$, $p<0.0001$), including in all areas of the MPA, i.e. reefs (R), next to reef (NR) and off-reef (OR) (Figure 19 and 20, Table 12; Dunn: all p<0.0001).

Within the MPA, crab CPUE varied significantly with habitat (Figure 20; Kruskal-Wallis: $H = 16.96$, $p = 0.0002$) and was significantly lower in R than in NR and OR (Dunn: p = 0.04 and p<0.0001, respectively), the latter having similar crab CPUE (Table 12; Dunn: p = 0.89).

Table 12. Mean shore crab CPUE (crabs/pot/day) in the MPA and in the non-MPA, as well as in three areas in the MPA: reef (R), next to reef (NR) and off-reef (OR). Significant differences from non-parametric Kruskal-Wallis and Dunn tests (p<0.05). $^+$ number of stations and * number of crabs.

		All			**Female**			**Male**		
Area	Significant difference	N^+	CPUE	SE	N*	CPUE	SE	N*	CPUE	SE
R	NR, OR, Non-MPA	64	0.37	0.09	6	0.04	0.02	45	0.33	0.08
NR	R, Non-MPA	48	0.89	0.18	10	0.11	0.05	74	0.78	0.15
OR	R, Non-MPA	99	1.30	0.18	44	0.21	0.05	239	1.09	0.15
MPA	Non-MPA	211	0.92	0.10	60	0.14	0.03	358	0.79	0.09
Non-MPA	MPA, R, NR, OR	170	2.46	0.16	169	0.47	0.06	735	1.99	0.13

Relative to substrate, crab CPUE was significantly different between substrates (Figure 21; Kruskal-Wallis: $H = 22.14$, $p<0.0001$) being lower in hard-boulder substrate (H) than the other three substrates (Dunn: all p<0.0001): gravel (G), mixed (M) and sand (S), which had similar crab CPUE (Dunn: all p>0.05).

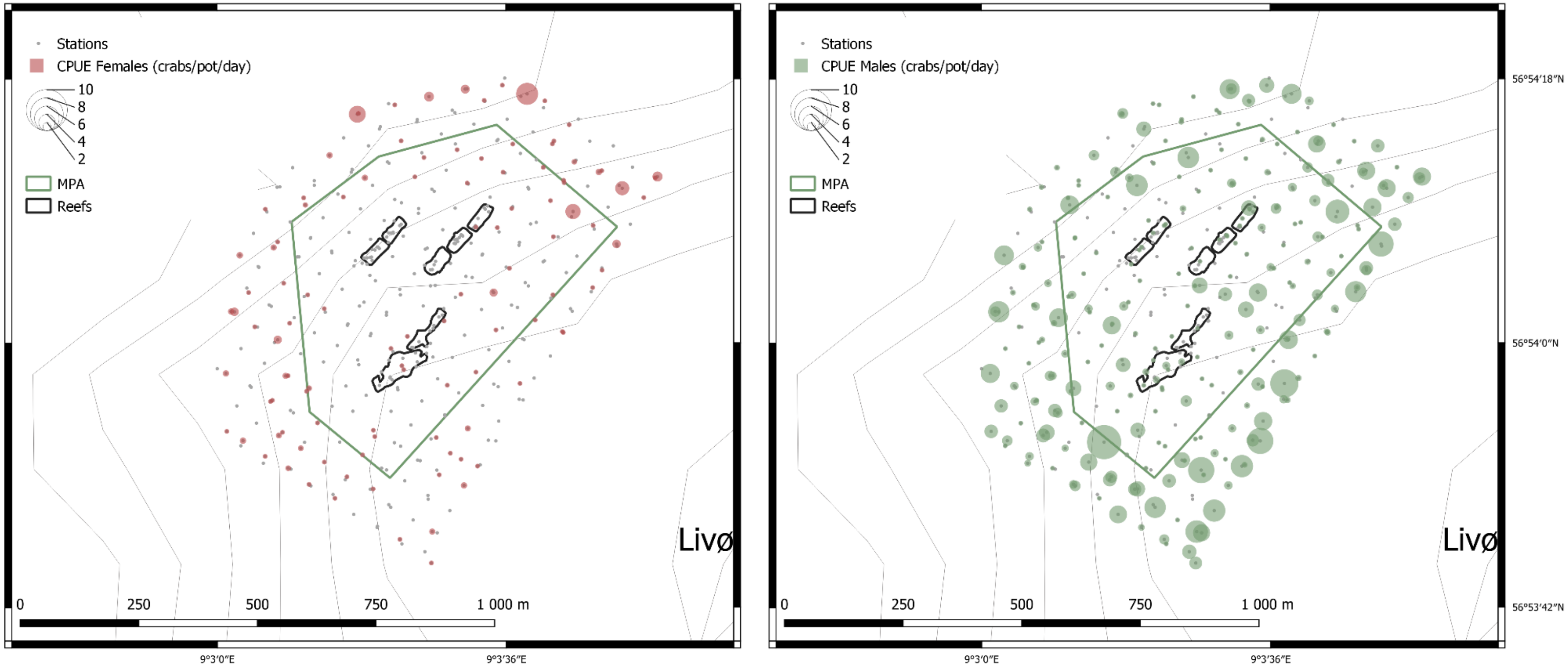

Figure 19. Relative reproductive potential index (RRP, top left), total spawning potential (ISP$_t$, top right), relative spawning potential (ISP$_r$, bottom left) and area spawning potential (ISP$_a$, bottom right) relative to size (carapace length) for the MPA (red), non-MPA (green) and control fished site (blue). Refer to the text and section 2 for a definition of the indices.

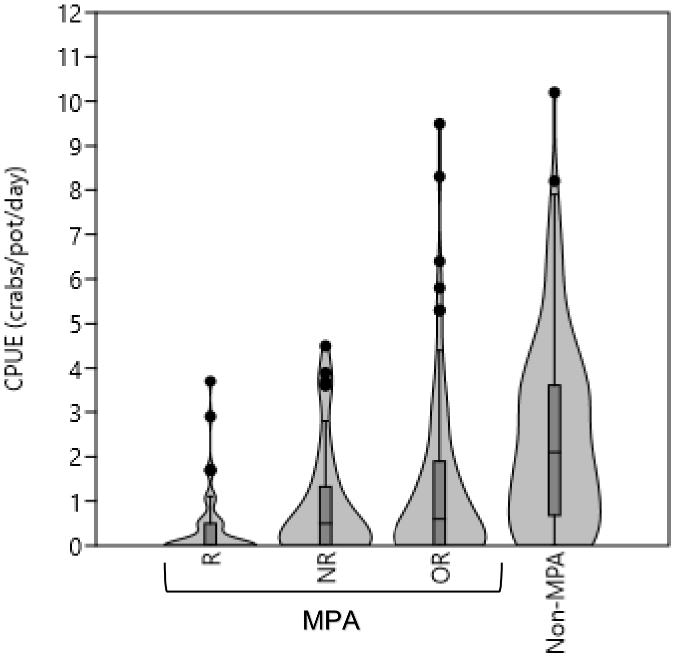

Figure 20. Violin and box plots of crab CPUE (crabs/pot/day) in the MPA on reefs (R), next to reef (NR) and off-reef (OR) and non-MPA with median, quartiles and outliers. Violin plot shows kernel density (continuous histogram) ranging from minimum to maximum values. CPUE distribution is non-normal and variance unequal (Anderson-Darling test, A2 = 22.09, p<0.0001; and Levene test, F = 45.45, p<0.0001).

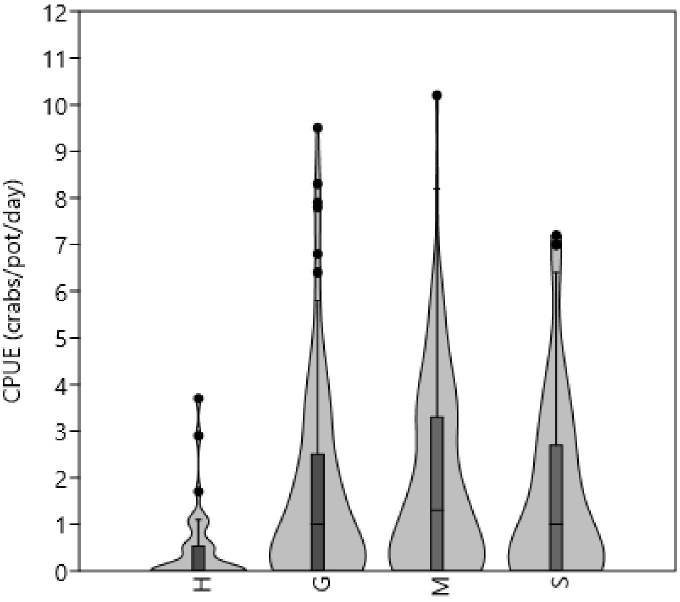

Figure 21. Violin and box plots of crab CPUE (crabs/pot/day) according to substrate: Hard-boulders (H), grave (G), mixed gravel-sand (M) and sand (S).

Crab CPUE was affected by lobster abundance, being negatively correlated and decreasing with increasing lobster CPUE (Figure 22; Zero inflated Poisson regression model selected relative to Poisson, negative binomial and zero inflated negative binomial using the Akaike information criterion corrected for small sample sizes AICc: X^2 (1, N = 380) = 170.06, $p<0.0001$, r^2 = 0.41).

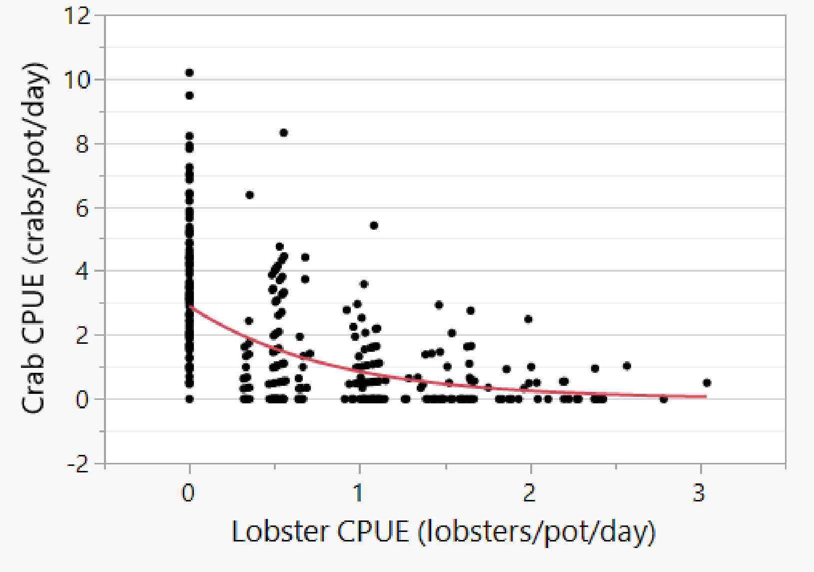

Figure 22. Zero inflated Poisson regression model between crab CPUE (crabs/pot/day) and lobster CPUE (lobsters/pot/day): X^2 (1, N = 380) = 170.06, $p<0.0001$, r^2 = 0.41.

2.9.2 Discussion

Shore crab *Carcinus maenas* abundance (i.e. CPUE) was significantly lower for females than males. However, it cannot be distinguished if such observation reflects a sex-specific spatial segregation of shore crabs, such as observed by Abelló et al. (1997) in Kerteminde Fjord where females only dominate the population in spawning grounds, or a reduced catchability of female shore crabs by the pots used in the survey.

No target fishing of shore crabs is known to occur in and around the MPA, and thus shore crab distribution should not be influenced by MPA protection from fishing. However, shore crab abundance was significantly lower in the MPA than in the adjacent non-MPA. Shore crab abundance was also significantly lower on reefs and hard-boulder substrate, which occur almost exclusively in the reefs, than surrounding non-reef areas either inside or outside the MPA.

Shore crabs are an extremely adaptable species, able to tolerate a wide range of environmental conditions, prey a upon a large variety of organisms, and occupy diverse habitats, including rocky shores (e.g. Klassen and Locke, 2007; Ens et al., 2022). However, shore crabs can favour sheltered areas protected from strong wave action and avoid high-energy sites (e.g. Hampton and Griffiths, 2007) and thus lower abundances in the reefs may result from higher wave exposure in the shallower reefs relative to non-reef areas since. On the other hand, shore crabs are known to suffer increased mortality and have agonistic interactions with adult American lobsters *Homarus americanus* (e.g. League-Pike et al., 2009; Rayner, 2018), a close relative of the European lobster *Homarus gammarus*. Similar negative interactions can be reasonably expected between shore crabs and European lobsters and shore crabs may avoid high lobster abundance areas inside the MPA, on and around the reefs, as well as avoid entering pots already containing lobsters. Thus, explaining the decrease in shore crab abundance with increasing lobster abundance (CPUE), the latter which was higher in the MPA than in the non-MPA and highest in reefs and next to reef areas (Section 5). The reverse may also happen where high crabs number of crabs reduce the catchability of lobsters in the pots (Rayner, 2018). Lower crab abundance in the MPA thus likely results from a combination of negative effects of reef habitat and hard-boulder substrate and/or agonistic interactions with lobsters.

2.10 Conclusions

Despite the caveats described in Section 2, which reduce the discriminant power of this study, significant positive effects of the Livø artificial stone reefs and/or MPA (LSR-MPA) on its lobster population were observed after only 3 years since its implementation, generally higher density, more abundant and of larger size in the artificial reefs and inside the MPA, with significant spill-over to adjacent non protected areas (Table 13).

The lobster density and abundance in the LSR-MPA were 2.1 to 2.4 times higher than non-MPA areas only 3 years after implementation, which is of similar magnitude to the 2.5 times increase in lobster abundance within 4 years of the establishment of MPAs in the UK and Norway (Hoskins et al., 2011, Moland et al., 2011a,b; Kleiven et al., 2019). Lobster abundance (CPUE) in the MPA and even the non-MPA were significantly higher (9 and 4 times, respectively) than what was observed across the entire Limfjorden in similar substrates. In addition, lobster size and reproductive potential were significantly larger in the MPA relative to control fished sites, an effect that spills to surrounding areas. The MPA thus hosts a high abundance of large lobsters, rare elsewhere, which have a significant disproportional reproductive value (Tully et al., 2001; Agnalt, 2007; ICES, 2003). The reproductive contribution of the MPA, excluding spill-over effects on surrounding areas, was estimated to be equivalent to a fishing ground 4 times its size.

Such rapid increases in lobster abundance and size in the LSR-MPA, have been described in other MPAs to result from increased survival, higher retention and limited export of lobsters (Hoskins et al., 2011; Moland et al., 2011a,b; Huserbråten et al., 2013; Kleiven et al. 2019), but also from large lobsters migrating into the MPA (Thorbjørsen et al., 2018). From observations in other MPAs, the positive effects of the LSR-MPA can be expected to continue, leading to long term changes (over >10 years) in the lobster population (Moland et al., 2013a,b; Fernandez-Chacon et al., 2020; 2021), i.e. the lobster population in the LSR-MPA likely has not reached an equilibrium and further increases in abundance and size are to be expected.

Estimates of the European lobster population in the entire Limfjorden are not available and thus the absolute population estimates obtained here for the LSR-MPA cannot be directly evaluated in the context of the entire Limfjorden lobster population. Nevertheless, relative indicators such as catch rates (CPUE), size, reproductive potential, and scale of spill-over to surrounding areas outside the MPA, all support a relevant positive impact of the MPA at least on the local Løgstør Bredning lobster population.

From a pure conservation perspective, considering the scale of lobster movement and spill-over from the MPA, habitat and fishing protection effects on the lobster population provided by LSR-

MPA would be more effective with a larger MPA. The findings from this study support the implementation or restoration of stone reef habitats and small MPAs as valid and relevant tools in the conservation of lobster populations in the Limfjorden, which can also play a significant role in the restoration and conservation actions in other Danish coastal systems. Further research can expand the knowledge on artificial reefs and small MPAs, how best to implement them, and what role they can play for the conservation and management of lobster populations, namely in coastal areas like Kattegat and Lillebælt, where lobster fishing pressure has increased significantly in recent years.

The main results and conclusions of the study describing positive effects on the lobster population from the artificial reef habitat and/or MPA fishing protection are summarized in tables 14 and 15 and presented here:

1. Higher lobster density and abundance in the MPA than in non-MPA site:
    a. Total density 2.1 times higher than in non-MPA.
    b. Harvestable density (i.e. > MLS) was 3.3 times higher than in non-MPA.
    c. Abundance (i.e. CPUE) was 2.5 times higher than in non-MPA.
    d. Decreases with distance to MPA boundary.

2. Higher lobster abundance in reefs:
    a. Higher in reefs (including next to reef) than in off-reef areas, but only for males.
    b. Decreases with distance to reefs.
    c. No difference between reefs of different stones and structure.
    d. Higher in hard-boulder and gravel substrates.

3. Significant mobility and spill-over to adjacent areas:
    a. Lobster moved several 100 up to 1,500m within a few days to few week.
    b. Lobsters migrated into and out of the MPA, including from reefs.
    c. 7.9% of all MPA lobsters estimated to be caught outside the MPA by a single fishing boat in 2021.
    d. Spill-over from MPA leads to larger sizes and reproductive potential in adjacent non-MPA than control fished site (see below).

4. Lobster size was larger in the MPA:
    a. Females and males were larger in the MPA than in the control fished site.
    b. Only males were larger in the MPA than in adjacent non-MPA: sex-specific MPA impact on survival/fishing mortality.
    c. Larger adult harvestable fraction: 58% in MPA, but only 18% in control fished site.

5. Reproductive and spawning potential is higher in the MPA:
    a. MPA reproductive potential was twice the non-MPA and 4 times the control fished site.
    b. 88% of spawning potential in the MPA from adult harvestable lobsters >MLS.
    c. In the control fished site, only 20% of spawning potential from lobsters >MLS.
    d. MPA equals the spawning potential of a fished area 4 times its area.

6. Shore crab abundance was lower in the reefs and MPA than in adjacent non-MPA site:
    a. Negative effects from reef habitat and hard-boulder substrate.
    b. And/or agonistic interactions with lobsters, which were more abundant in the reefs and MPA.

Table 13. Summary of effects of artificial reef habitat and/or MPA protection on the lobster population relative to the non-MPA comparison site and the control fished site. + and - symbols indicates higher or larger and lower or smaller, respectively, between different habitats and sites. m is for male lobsters and f for female lobsters.

Lobster	Reef	Off-reef	MPA	Non-MPA	Control	Process	Comments
Density			+	-		Habitat + Protection Effects: Survival Retention Immigration	Total: MPA = 2.1x of non-MPA Harvestable: MPA = 3.3x of non-MPA MPA = 5.5% of 2021 landings
CPUE	$=^{f} / +^{m}$	$=^{f} / -^{m}$	+	-			MPA = 2.4x non-MPA Higher in reefs and hard boulder Decreases with distance to reefs/MPA
Size	=	=	$=^{f} / +^{m}$	$=^{f} / -^{m}$	-		
Size at first catch (L_{50})			^{f} 84.8 ^{m} 94.5	^{f} 84.1 ^{m} 80.4	^{f} 76.8 ^{m} 75.5		Carapace length (mm)
Size of larger 5% (L_{95})			^{f} 109.2 ^{m} 124.9	^{f} 109.8 ^{m} 106.7	^{f} 93.2 ^{m} 101.8		Carapace length (mm)
Harvestable Fraction			17.9%	34.6%	57.6%		> MLS, no ovigerous females
Reproductive Potential			+	+ / -	-		From size: MPA = non-MPA or 2x Control From Abundance: MPA = 2x non-MPA or 4x Control MPA = Fished area 4x larger Spawning potential > MLS: MPA 88%, Control only 20%
Sex	Males 2 x females					Catchability? Population?	In other fishing grounds is approximately 1:1
Spillover	Similar		+			Mobility	Up to 1,500m in 1 to 4 weeks Leads to larger size + reproductive potential in non-MPA 7.9% of MPA lobsters fished in non-MPA, 1 boat in 2021 6.6% >MLS = 52,400 kr. in 2021 for 1 boat

Table 14. Summary of effects of artificial reef habitat and/or MPA protection on the crab population relative to the non-MPA comparison site.

Shore Crab	Reef	Off-reef	MPA	Non-MPA	Process	Comments
Abundance	-	+	-	+	Habitat? Lobster interaction? Catchability?	Highly skewed sex ratio Lowest in reefs + hard boulder Lower in MPA, but no fishing protection Increases with distance to reefs/MPA Decreases with lobster abundance

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3. The effects of the Livø stone reef on fish and biodiversity

Using baited remote underwater video systems to compare fish abundance and biodiversity between artificial reef areas and control areas

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3.1 Summary

Coastal habitats are degraded in numerous areas, both internationally and along most Danish coastlines. The degradation is caused by many factors, including eutrophication, fishing, resource extraction and land reclamation. In 2017, a geogenic reef consisting of 12,000 m^2 rocks was established in Løgstør Bredning, situated in the central part of the Limfjorden, which is an estuarine area in northern Denmark. A favourable nutrient balance was the overarching objective of the project; however, a rocky reef may also have positive effects on fish abundance and biodiversity. To scrutinize the issue, this study deployed baited remote underwater video systems (BRUVS) to compare fish abundance and biodiversity in reef areas and suitable control areas, the latter having sediments dominated by sand and mud. BRUVS were used to detect species and produce a measure of relative abundance termed MaxN. In addition, the environmental status of the marine area (Løgstør Bredning) was investigated by downloading and summarising existing environmental data originating from the period 2000 – 2020. Applying European Union thresholds, the environmental status was assessed using chlorophyl a concentration and the maximum water depth with significant growth of eelgrass (*Zostera marina*). Data revealed statistically significant differences in biodiversity and community structure between reef areas and control areas. Moreover, abundances of economically and ecologically important species, including European lobster (*Homarus gammarus*) and shore crab (*Carcinus maenas*), differed between reef areas and control areas. Specifically, the average ratio between $MaxN_{reef}$ and $MaxN_{control}$ for European lobster was 6.5, indicating higher abundances of European lobster in the reef areas. In contrast, the average ratio between $MaxN_{reef}$ and $MaxN_{control}$ for shore crab was 0.5:1, indicating lower abundances of shore crab in the reef areas. Three diversity indices were elevated in the reef areas compared to the control areas. Specifically, the indices species richness, Shannon-Wiener and Pielou’s Evenness were significantly higher in the reef areas, indicating elevated diversity associated with the hard substrate, about four years after reef deployment. Multi-variate species analyses revealed that European lobster, caridean shrimp (*Caridea* sp.) and goldsinny wrasse (*Ctenolabrus rupestris*) were strongly associated with reef areas. Data on the environmental status of the marine area revealed a poor ecological status according to the thresholds developed for the European Union Water Framework Directive. Our study suggests that the characteristics of marine habitats affect the biodiversity and abun-

dances of economically and ecologically important species. These habitat effects were documented despite the poor environmental status of the marine area, indicating that habitat conservation and restoration may yield positive outcomes even in degraded aquatic environments.

The present study was carried out in 2021 and revealed significant effects of a man-made rocky reef on fish abundance and biodiversity in the Limfjorden. Fisheries assessments have, however, been carried out for decades in the area. Among others, the assessments have indicated steadily decreasing catches of plaice (*Pleuronectes platessa*), Atlantic cod (*Gadus morhua*) and European eel (*Anguilla anguilla*) since the early 1900s. In the early 1990s, a regime shift was detected among important benthic species, resulting in consistently low abundances of several fish species. There is no indication that historic abundances of benthic fish species are recovering, as revealed by an ongoing citizen science-based project including survey data from more than 15 years. In contrast, abundance of European lobster started increasing in the early 2000s, and the species is now dominating catches in many areas. As a perspective, it is proposed that several factors can be addressed to start restoring historic fisheries in the Limfjorden, including 1) elimination of severe oxygen depletion, 2) adjusting and regulating fishing methods that directly harm the environment, 3) regulation of severe avian predation on juvenile fishes, 4) establishment of no-take areas in conjunction with restoration of historic eelgrass coverage as well as geogenic and biogenic reef areas, and 5) assessment of the connectivity and inflow of juveniles (e.g., fish eggs and larvae) from surrounding marine areas (e.g. the North Sea) and the ongoing fishing in those areas. It is important to note that these five factors are based on a preliminary assessment and further studies are needed to elucidate the effects of each factor and advance future marine management.

3.2 Introduction

The removal of stone reefs and boulders in coastal areas for the construction of harbours, piers and coastal protection has been widely documented in Denmark (Dahl, 2003). Removal of large boulders increases the depth below the water surface and consequently influences light penetration, which may reduce macro algae growth (Støttrup et al., 2014; Dahl, 2003). As well as providing suitable conditions for the growth of algae, rocky reefs also provide a hard substrate favourable to many benthic organisms and shelter as well as spawning and nursery areas for a range of fish species. Therefore, the removal of boulders often causes a loss of important habitat or a reduction in complexity of the ecosystem (Hunter & Sayer, 2009). After the construction of a rocky reefs at Læsø (northern Kattegat Sea), it was reported that macro algae had resettled in the area to the benefit of higher numbers of fish (Støttrup et al., 2014; Dahl, 2003).

In the Limfjorden (northern Denmark), the Løgstør Bredning rocky reef project was commissioned by the Danish parliament, aiming to utilise artificial reefs as a mitigation tool to reduce eutrophication problems (DHI, 2016). To this end, an artificial rocky reef was established in 2017 and assessed in relation to mitigation of eutrophication problems. In addition, the rocky reef could have positive effects on marine biodiversity and fish abundances, however, to date, it remains unknown if the established rocky reef has influenced the mobile macro fauna (e.g., fish) in the marine area.

The aim of this study was to assess the effects of the Løgstør Bredning rocky reef on fish abundance and biodiversity using baited remote underwater video systems (BRUVS). Previous studies investigating the effects of various marine habitats utilising BRUVS have demonstrated that species, such as Atlantic cod (*Gadus morhua*) and Goldsinny wrasse (*Ctenolabris rupestris*), may benefit significantly from increased coverage of rocky structures (Rhodes et al., 2020). On this basis, we predicted that abundance of several local species would differ between Løgstør Bredning rocky reef and suitable control areas without reef structures.

The ecological community in the Limfjorden has undergone a shift from fauna dominated by demersal fish species such as flounder, plaice, European eel, eelpout, whiting and shorthorn sculpin towards communities dominated by smaller fish species, such as sprat and herring, as well as jellyfish, starfish, and shore crab (Hoffmann, 2005; Jacobsen, 2003; Tomczak et al., 2013a). This is confirmed by recent investigations (2017 – 2018) showing that the depauperate fish community persists and is often dominated by small species including various sticklebacks (*Gasterosteidae* spp) and gobies (*Gobidae* spp) (Støttrup et al., 2020). The changes in regime have largely been driven by increased nutrient input, high fishing pressures and possibly climatic changes (Tomczak et al., 2013). In particular, the large nutrient inputs increase the production of phytoplankton (micro algae) and cause poor visibility (Riisgård et al., 2012). As a result of nutrient inputs, the depth at which eelgrass can grow is greatly reduced (Krause-Jensen et al., 2011). Degrading phytoplankton may also cause oxygen depletion, as often observed in the Limfjorden during the summer and autumn months (Riisgård et al., 2012). These changes have detrimental effects on most benthic fauna.

The aim of the project is:

1. Use BRUVS to document the abundance of fish and biodiversity at the Løgstør Bredning rocky reef and at suitable control areas, characterized by sandy or muddy sediments.
2. Statistically test the BRUVS data covering reef and control areas to assess whether the established reef affects marine biodiversity and species abundances.

BRUVS are increasingly used for habitat studies and have been used on a wide geographic scale, offering the flexibility to adjust the setup to the specific ecosystem. An advantage of using BRUVS over most other methods is that BRUVS do not cause detrimental effects to the ecosystem or habitat destruction (Rhodes et al., 2020; Wilms et al., 2021). No fish are caught and killed when BRUVS are used for sampling. This has further advantages because sampling can be completed without permits from the fishing authorities and agencies. In addition, sampling bias may be reduced compared to other methods (Shoup & Ryswyk, 2016; Šmejkal et al., 2015), and the use of BRUVS is often considered a cost-effective approach (Mallet & Pelletier, 2014; Schmid et al., 2017).

3.3 Materials and methods

3.3.1 Study location and environmental status

The Limfjorden is an estuarine area connected to the North Sea in the west and to the Kattegat Sea to the east in northern Demark. It is a relatively shallow basin with an average depth of 4.5 m (Wiles et al., 2006). Salinity levels in the study area typically range between 23-33 PSU, and the tidal amplitude is usually between 10 – 20 cm. The strong west – east prevailing winds causes high salinity water to enter the Limfjorden from the North Sea, however the system is also receiving a freshwater inflow of 2.6 km^3 annually (Riisgård et al., 2012).

The artificial rocky reefs were established in 2017 and comprise three distinct reefs (RA, RB and RC; Figure 23). The rocky reefs were deployed at water depths (from the water surface to the seabed) ranging between 3.5-5.7 m (DHI, 2016). The study areas were in the Løgstør Bredning of the Limfjorden.

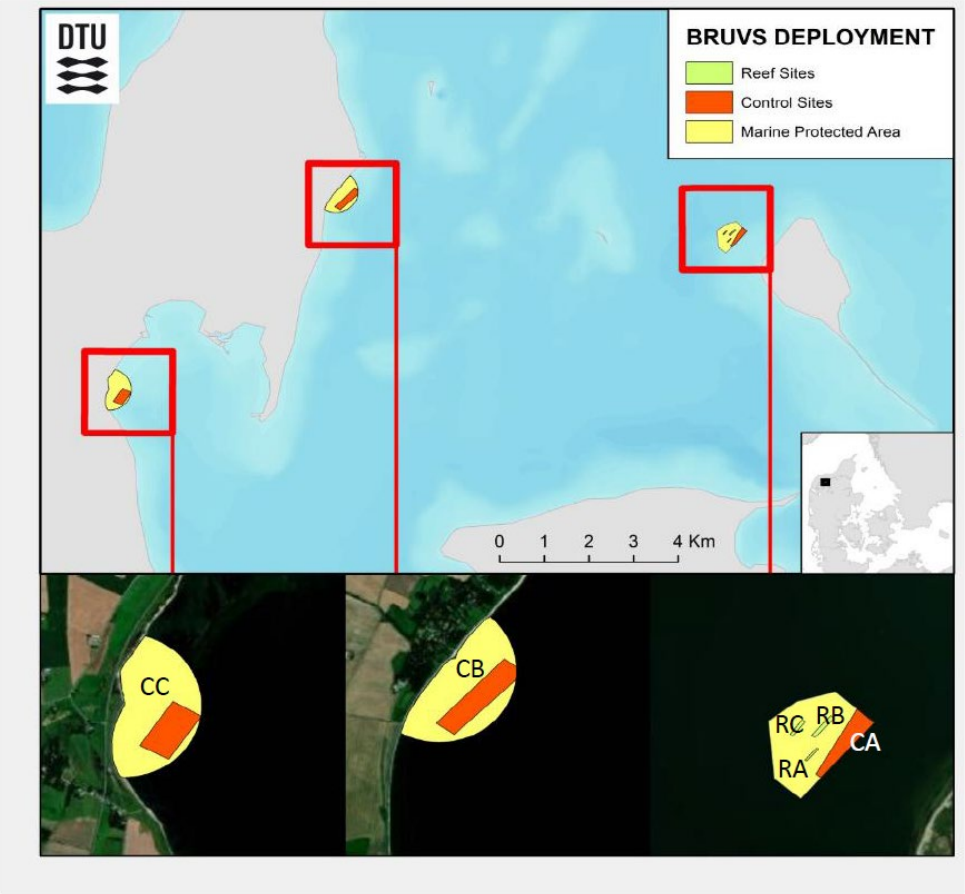


Figure 23. The study location was Løgstør Bredning in the Limfjorden with the six sampling locations indicated. Baited remote underwater video systems (BRUVS) were deployed in control areas CA, CB and CC (red markings) and in reef areas RA, RB and RC (green markings). All BRUVS deployments were completed inside marine protected areas (yellow areas). Therefore, reef and control areas were subjected to the same protection level (i.e., no fishing allowed).

3.3.2 Study design and camera system set-up

BRUVS deployments were carried out from research vessels during the months of August – October 2021 during daylight hours. BRUVS were slowly lowered onto the seabed at pre-determined locations for a comparison of two distinct habitat types: 1) rocky reef areas and 2) suitable control areas with sandy and muddy sediments (Figure 23).

The deployments were comprised of equal amounts of reef areas and control areas for balanced statistical comparisons. The reef area and control area BRUV deployments were subdivided between six separate sites, covering three reef (RA, RB and RC) and three control (CA, CB and CC) areas (Figure 23). Control area A (CA) was located approximately 200 m west of the nearest reef within the Løgstør Bredning rocky reef marine protected area (MPA) (Figure

23). The two remaining control areas (CB and CC) were located 7-9 km in the eastern direction, also situated inside MPAs (Figure 23). Therefore, reef and control areas were subjected to the same protection level (i.e., no fishing allowed). This ensured that the specific effects of the deployed rocky reef could be detected and assessed.

All control areas were selected based on the water depth, seabed substrate, and the presence of a MPA to match the conditions at the reef areas. Specifically, existing seabed mapping was used to select the locations, followed by subsequent underwater recordings to confirm the absence of rocks and the presence of sandy and muddy sediments in the control areas. Blue mussels (*Mytilus edulis*) were occasionally present on the seabed but maintained at coverages < 10%. The water depths of the control area deployments matched the water depths of the reef area deployments for an unbiased comparison between the various areas. The deployment water depths ranged between 1.9-4.6 m.

Euclidean distances between deployments were actively maximized, and temporally overlapping camera deployments were only allowed given a minimum distance greater than 100 m apart to minimize the probability of attracting fish from adjacent deployments by the bait (Hesse et al., 2016).

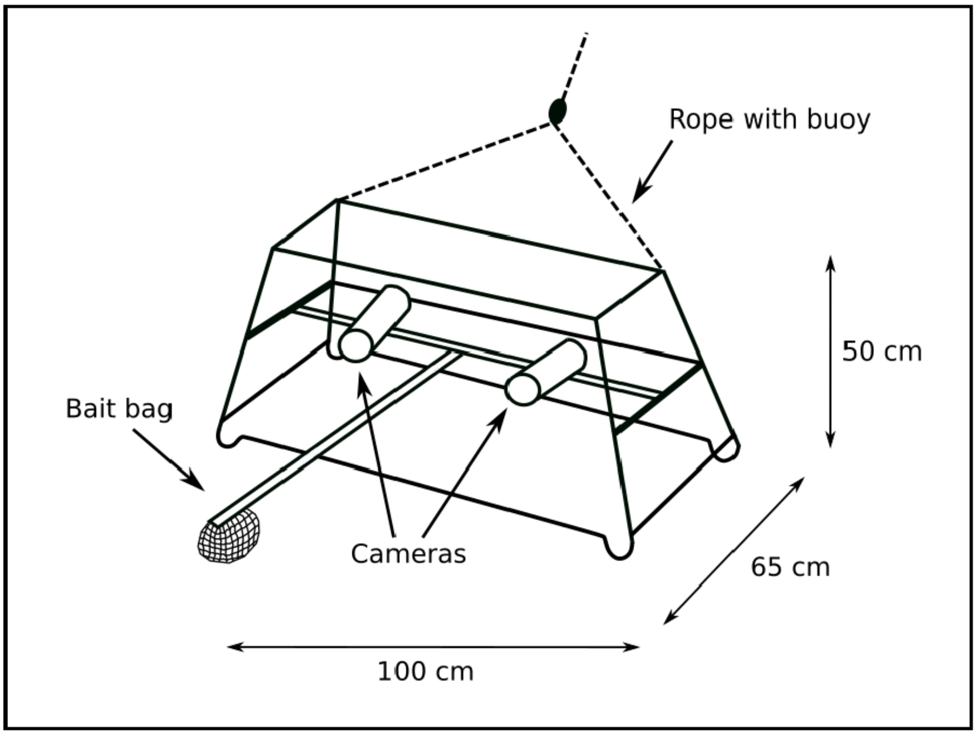


Figure 24. Stereo baited remote underwater video system (BRUVS) used in the present study. Image originates from a previous study (Stöhr, 2021).

Following a previous study (Stöhr, 2021), BRUVS units were composed of a metal frame (100 cm x 65 cm x 50 cm; Figure 24) with a rope, anchor and buoy attached. Two SONY FDR-X3000 cameras powered with an additional external battery (ANKER PowerCore 10000 mAh), situated in a watertight SeaGIS camera housing, were horizontally mounted on the metal frame 30 cm above the seabed and 60 cm apart at an angle of 8 ° convergence. A horizontal metal bait arm (100 cm long) was attached to the frame at the midpoint between the two cameras (Stöhr, 2021). A mesh bait bag containing 500 g of chopped Atlantic herring (*Clupea harengus*) was attached to the end of the bait arm (Figure 24; Stöhr, 2021).

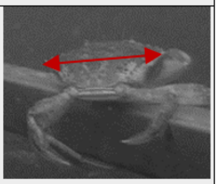
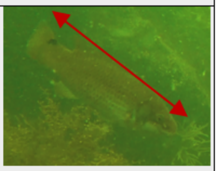
Studies comparing the effect of the total soak time of underwater recordings lasting between 30 and 90 min on species richness did not conclude any significant difference, however the accuracy of the data collected from the recordings increased with soak time (Gladstone et al., 2012). A total soak time of 60 min was used consistently throughout the present study, with an additional 5 minutes at the beginning for settlement of disturbed sediments. For each deployment, the bait bag was exchanged for a new bait bag to ensure consistent data collection, because repeatedly used bait bags would have less odour causing diminishing attraction (Hesse et al., 2016).

3.3.3 Video analyses

In total, 90 BRUVS deployments were distributed equally across the three reef areas and the three control areas (i.e., 15 deployments in each area). All recordings were analysed using the SeaGIS Event Measure software, developed specifically for BRUVS. Following previous studies (Rhodes et al., 2020; Stöhr, 2021), all mobile fauna was identified to species level where feasible, or to the lowest taxonomic level possible, and subsequently quantified. Mobile fauna covered larger crustaceans, including European lobster and shore crab, and several species of small fish (e.g., two spotted goby; *Pomatoschistus flavescens*). Baltic prawn (*Palaemon adspersus*) is considered the most abundant shrimp species in the Limfjorden, however, based on video recordings, it is largely impossible to distinguish the species from other shrimp species (including rockpool shrimp *Palaemon elegans* and common shrimp *Crangon crangon*). In the present study, shrimp species are therefore reported as Caridean shrimp (*Caridea* sp.), which includes several shrimp species.

Mobile fauna was quantified using MaxN, which describes for each species the maximum number of individuals per video frame within the total analysis period of 60 min (Priede et al., 1994). The use of MaxN eliminates potential bias, which would otherwise be introduced by making repeated counts of individuals entering, and re-entering, the camera field of view within the total analysis period. Therefore, MaxN gives a more suitable and conservative measure of species abundance. For each deployment, data were collected on: A) species present, B) MaxN, and C) water depth using the boats' echo-sounder. Like previous studies (Rhodes et al., 2020), deployments were considered unsuccessful and discarded when 1) cameras failed to record for the full 60 minutes, 2) the camera frames (Figure 24) did not land horizontally and were pointing towards the water surface, or 3) the camera field of view was severely obstructed due to the presence of macro algae or rock.

Table 15. Latin name, common name, size range (cm) and mean length measurements (cm) for key species from the event measure software. Shore crabs were measured by the widest points of the carapace, and all fish were measured by the total length, as indicated by the red arrows.

Carcinus maenas	Shore crab	1.9 – 12.4	4.7	
Ctenoblabris rupestris	Goldsinny wrasse	10.8 – 14.0	12.3	

Body length measurements of detected organisms were recorded using the Event measure software developed by SeaGIS (Bacchus Marsh VIC 3340, Australia) when feasible. To measure the body length of recorded species using stereo cameras, the mobile fauna must be in the field of view for both the left and right cameras simultaneously (Figure 24). However, on several occasions, mobile fauna was only visible in one of the two cameras, meaning that not all MaxN entries have a corresponding body length measurement. Table 15 provides a summary of the body length measurements for two species that were recorded frequently.

3.3.4 Statistical analysis

Analyses of variance (ANOVA) were used to test the effect of deployment type (i.e., reef areas versus control areas; Figure 23) on the abundances of the species European lobster and shore crab.

Univariate analysis was used to determine biodiversity indices at all 90 deployment sites. The following three diversity indices were calculated:

1. Species richness, which is a measure of the number of unique species identified per successful deployment. This is based on the consideration that areas with an elevated number of species may represent a richer community (Christianen et al., 2017; Wilhelmsson et al., 2006).
2. Shannon-Wiener index, which accounts for both the number of species and their proportional abundances (Furness & Unsworth, 2020). More species and less variation in species abundance are associated with an elevated Shannon-Wiener index.
3. Pielou's evenness index, which is a measure of how even the counts of individual species are. Specifically, if all species in a community are equally common (equal abundance), Pielou's evenness index is elevated and will approach a value of 1 (Pielou, 1966; Fariñas-Franco et al., 2013; Lemieux & Cusson, 2014).

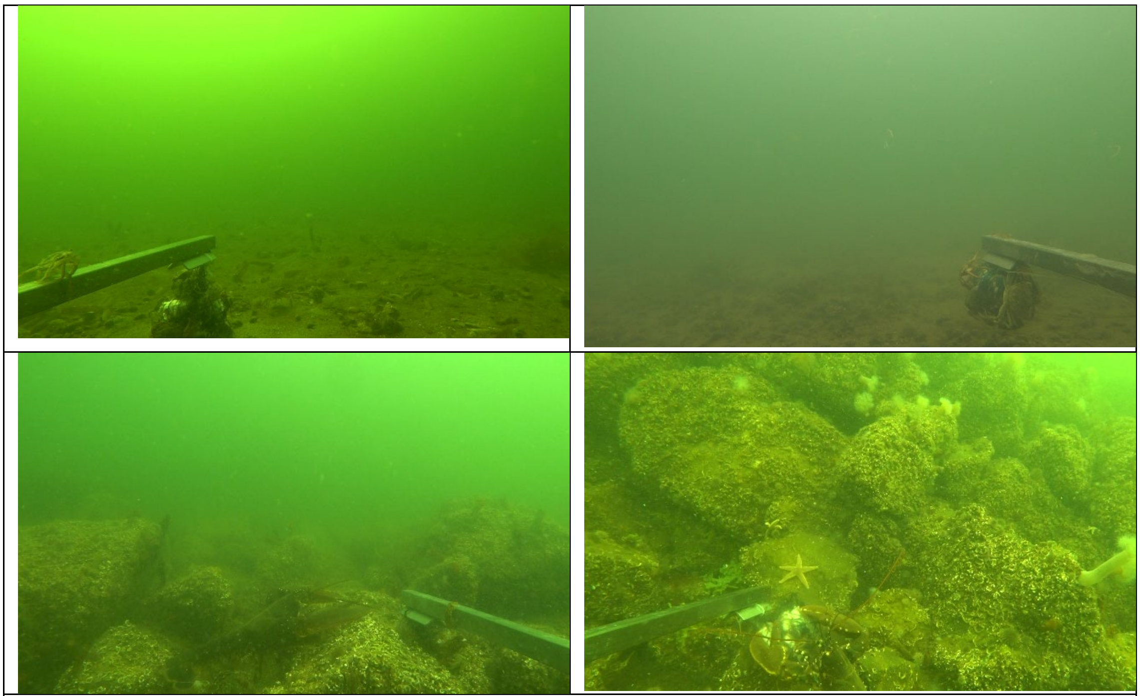
Multivariate statistical analyses were used to test the difference in community composition by grouping the sites into reef areas and control areas. The analyses were carried out in R using the vegan package and applying an unconstrained ordination method termed non-metric multidimensional scaling (nMDS) with Bray-Curtis as the dissimilarity index (Faith et al., 1987) to calculate the distance matrix. Stress values indicate how suitable the reduced dimensions are at representing the multidimensional data and should ideally be less than 0.2, otherwise plots cannot be reliably interpreted (Clarke, 1993). With a *k* value of 2 (2 dimensions), the stress was too high (>0.21), therefore *k* was increased to 3, which resulted in a suitable stress decrease. A permutational multivariate ANOVA was used to test the difference in distance matrix between reef and control areas using reef/control ID as a factor variable, like previous studies (Oksanen et al., 2019).

To further investigate which species were causing the differences detected in the multivariate analysis, and their association with a group or combination of groups, indicator species analysis was undertaken with the *indicspecies* package (De Cáceres, 2020). Analyses were conducted using raw data without adjustments for possible variation in water temperature, oxygen content, visibility etc. Any variation in such variables was assumed to be similar across the examined areas (i.e., reef areas and control areas).

3.4 Results

Overall, 90 BRUV deployments were successful, and a total of 23 species taxa were observed and identified to species level during the video analyses (Table 16). The minimum number of unique species recorded within a single deployment was zero and the maximum was seven. Of the 23 species observed, 11 were fish species. The other species included large crustaceans such as European lobster and shore crab.

Figure 25 below shows four images of typical deployments from the Løgstør Bredning.



Top left and top right show typical control areas, dominated by sandy bottom substrate and shore crabs. Bottom left and bottom right show examples of camera deployments in the reef areas. Recorded species include European lobster, common starfish and sea anemone.

Figure 25. Examples of images recorded during the video analyses. All deployments covered 60 min of continuous video recording, in either control areas (top images) or reef areas (lower images). In total, 90 successful deployments were analysed.

A summary of the raw data counts is provided in Table 16.

Table 16. List of all species observed throughout the final 90 successful deployments (Latin and common names), Total control cumulative MaxN (Control), Total reef cumulative MaxN (Reef), overall total cumulative MaxN (Total) and the number of times the species appeared in a deployment (#Occur). Note that sea anemone and jellyfish species were detected, but disregarded in the analyses, because they are not considered mobile fauna.

Species Name	Common name	Control	Reef	Total	#Occur
Aphia minuta	Transparent goby	4	1	5	5
Asterias rubens	Common starfish	26	37	63	41
Aurelia aurita	Common jellyfish	75	86	161	58
Bolinopsis infundibulum	Common northern comb jelly	40	36	76	39
Carcinus maenas	Common crab	369	175	544	88
Caridea sp.	Caridean shrimp	14	117	131	37
Clupea harengus	Atlantic herring	48	52	100	8
Ctenolabrus rupestris	Goldsinny wrasse	0	11	11	10
Gobius niger	Black goby	41	149	190	63
Homarus gammarus	European lobster	6	39	45	38
Pleuronectidae sp.	Unidentified flatfish	5	0	5	4
Macropodia rostrata	Sea spider	0	1	1	1
Metridium sp.	Sea anemone	0	78	78	18
Mnemiopsis leidyi	Warty comb jellyfish	76	37	113	60
Tauruls bubalis	Longspined sea-scorpion	0	1	1	1
Phalacrocorax carbo	Cormorant	0	1	1	1
Phoca vitulina	Harbour seal	1	0	1	1
Pleuronectidae sp.	Unidentified flatfish	5	0	5	4
Pomatoschistus sp.	Sand goby	48	13	61	28
Pomatoschistus flavescens	Two spotted goby	3	0	3	1
Pungitius pungitius	Nine - spined stickleback	5	0	5	5
Syngnathus rostellatus	Lesser pipefish	3	2	5	5
Zoarces viviparus	Eelpout	0	2	2	2

To identify the differences between reef areas and control areas, the ratios between reef $\text{MaxN}_{\text{reef}}$ and $\text{MaxN}_{\text{control}}$ were analysed. A ratio >1 would indicate increased abundance in the reef areas, whereas a ratio <1 would indicate decreased abundance in the reef areas. The ratio $\text{MaxN}_{\text{reef}}$ and $\text{MaxN}_{\text{control}}$ for key species was A) European lobster 6.5:1; B) shore crab 0.5:1; C) goldsinny wrasse were not encountered in any control area, whereas a total cumulative MaxN of 11 was recorded in the reef areas. The raw data reveal higher numbers of European lobster and wrasse in the reef areas, and higher numbers of shore crab in the control areas (Table 16).

The results of the ANOVA for the key species, European lobster and shore crab revealed statistically significant differences between the reef and control areas ($p < 0.001$; Table 16 and 17). The bar graphs below (Figures 26 and 27) provide comparisons of the raw MaxN data for both European lobster and shore crab in control areas and reef areas. The total MaxN for the six survey sites is also used in the subsequent analyses. The results show significant differences in

MaxN between reef and control areas, both from the individual analysis of MaxN, and the species indicator analysis, which is highlighted in section 3.4.2.

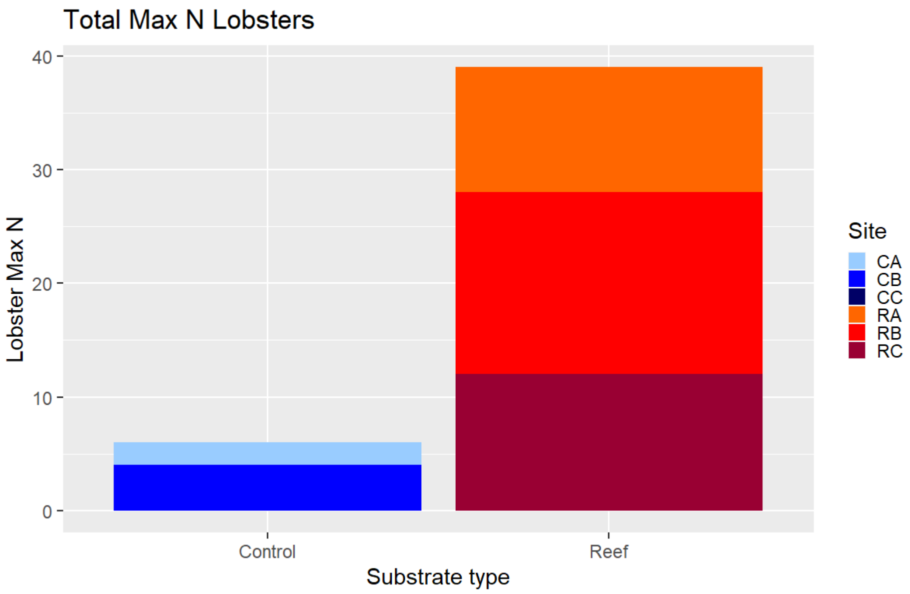


Figure 26. Raw data revealing total MaxN for European lobsters (Homarus gammarus) in control areas and reef areas. Colours on the legend correspond to the six individual deployment sites, CA = Control site A, CB = Control site B, RA = Reef site A, etc. Note that Control site C did not record any European lobster (MaxN = 0), explaining why only five sites are presented here. The data show elevated abundance of European lobster in the reef areas. Reef areas and control areas are outlined in detail in Figure 23.

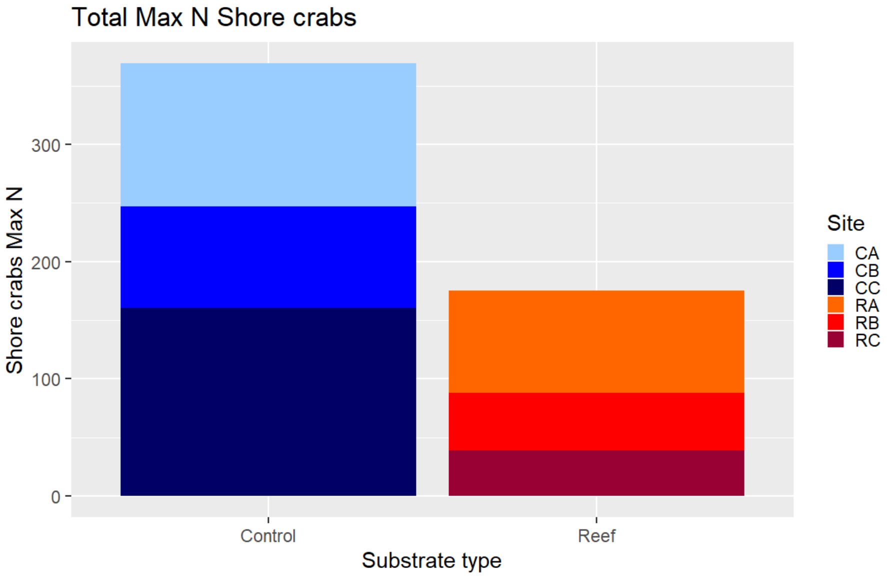


Figure 27. Raw data revealing total MaxN for shore crab (Carcinus maenas) in control areas and reef areas. Colours on the legend correspond to the six individual deployment sites, CA = Control site A, CB = Control site B, RA = Reef site A, etc. The data show reduced abundance of shore crab in the reef areas. Reef areas and control areas are outlined in detail in Figure 23.

Table 17. One–way ANOVA summary highlighting the effect of reef areas and control areas on MaxN of European lobster and shore crab. Df indicate degrees of freedom. The results provide statistical evidence of increased and decreased abundances of European lobster and shore crabs in reef areas, respectively.

Species	Dependent Variable	Df	Sum of squares	Mean of squares	F value	P value
Homarus gammarus	**Reef effect**	1	12.1	12.100	43.64	**< 0.001**
	Residuals	88	24.4	0.277		
Carcinus maenas	**Reef effect**	1	418.2	418.2	31.52	**< 0.001**
	Residuals	88	1167.6	13.3		

3.4.1 Community analysis

Species richness

From the total of 23 recorded species, an equal number of 17 unique species were observed in both the reef areas and control areas. However, a one-way ANOVA comparing species counts from individual BRUV deployments revealed significantly higher species richness in reef areas compared to control areas ($p = 0.0227$; Table 18; Figure 28A).

Shannon–Wiener diversity Index

The Shannon-Wiener diversity index can range between 0 and 5. Index values for the present study ranged between 0 and 1.8. A one-way ANOVA indicated significantly elevated Shannon-Wiener index in the reef areas ($p < 0.001$). The finding suggests increased diversity in the reef areas (Figure 28B).

Pielou's Evenness

A one-way ANOVA revealed that the reef areas were associated with elevated Pielou's Evenness index ($p < 0.001$) (Figure 28C). Specifically, values of evenness ranged between 0 and 1. A value close to 1 indicates similar abundances among the species identified, whereas values close to zero indicate dominance of certain species (e.g., shore crab). The finding suggests reduced dominance by certain species in the reef areas.

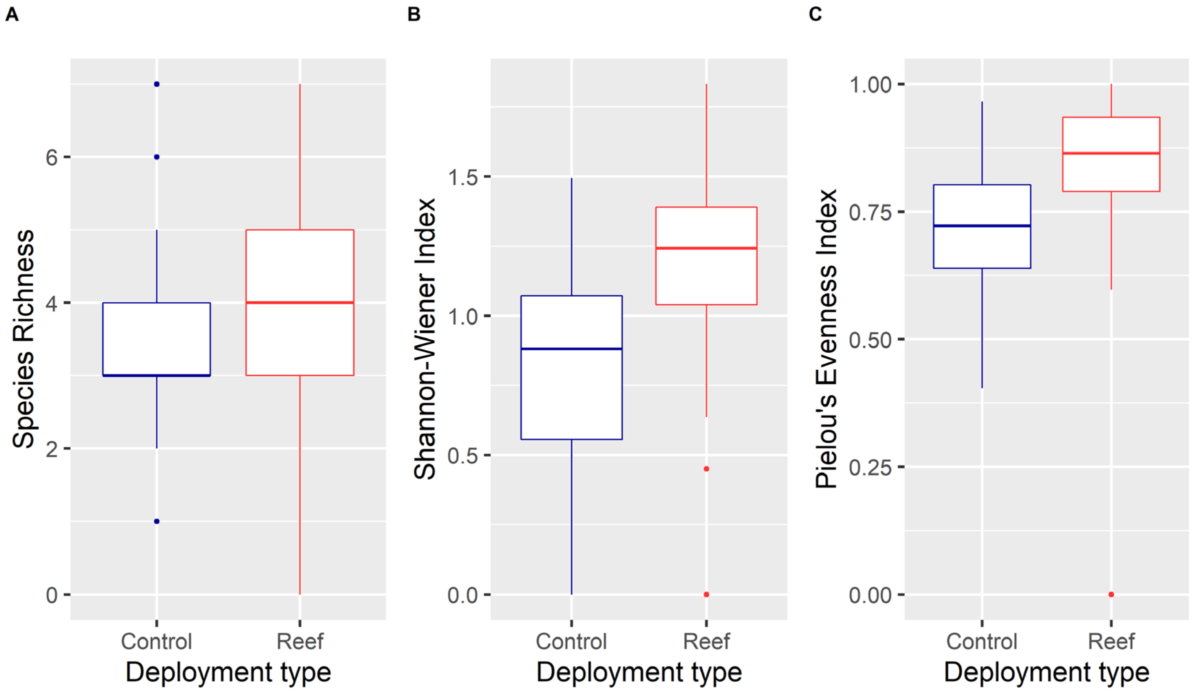


Figure 28. Box plot of biodiversity indices, A) Species richness, B) Shannon-Wiener index and C) Pielou's Evenness based on the raw data. Data from control areas are marked blue, and data from the reef areas are marked red. All three indices differed significantly ($p < 0.05$) between control areas and reef areas, indicating diversity differences between the two different benthic habitats. Collectively, the indices suggest elevated biodiversity in the reef areas.

Table 18. Biodiversity indices revealed by Species richness, Shannon- Wiener index, and Pielou's Evenness Index tested for reef effect using one-way ANOVA. Collectively, the indices suggest elevated biodiversity in the reef areas.

Diversity index	Dependent variable	Df	Sum of squares	Mean of squares	F value	P value
Species Richness	**Reef effect**	1	12.84	12.84	5.381	**0.0227**
	Residuals	88	210.04	2.387		
Shannon - Wiener	**Reef effect**	1	2.738	2.738	17.49	**<0.001**
	Residuals	88	10.87	0.124		
Pielou's Evenness	**Reef effect**	1	0.298	0.298	12.57	**<0.001**
	Residuals	82	1.943	0.024		

3.4.2 Multi-variate analysis of community composition

The results of the indicator species analysis further investigated the distribution of species among the different areas (Table 19). Two species of mobile fauna were strongly associated with reef areas, European lobster and Caridean shrimp, while goldsinny wrasse was linked specifically to reef site RC. In contrast, common periwinkle (*Littorina littorea)* was associated exclusively with control site CC. Finally, black goby (*Gobius niger*) was associated with all three reef sites and control site CA, while sand goby (*Pomatoschistus* sp.) was associated with all three control sites and reef site RA.

Table 19. Indicator species analysis showing species significantly associated with site groups RA, RB and RC (Reef sites) and CA, CB and CC (Control sites). Red highlighted rows show indicator species associated exclusively with reef sites. '#Occur' is the number of deployments where the species was present out of 90 deployments. 'A' is the specificity, which is the probability that a surveyed site belongs to the indicated group, since the species was found. 'A' is maximum when the species is only present at sites of this group. B describes the probability of finding the species at sites belonging to the group. 'B' is called fidelity, and it is maximum when the species occurs at all sites of the group.

Group	Species	#Occur	A	B	p value
RA, RB, RC, CA	*Gobius niger* (Black goby)	63	0.905	0.850	**0.001**
CA, CB, CC, RA	*Pomatoschistus* sp. (Sand goby)	28	0.918	0.417	**0.014**
RA, RB, RC	*Homarus gammarus* (European lobster)	38	0.867	0.711	**0.001**
RA, RB, RC	*Caridea* sp. (Caridean shrimp)	37	0.893	0.533	**0.027**
RC	*Ctenolabrus rupestris* (Goldsinny wrasse)	10	0.636	0.400	**0.005**
CC	*Littorina littorea* (Common periwinkle)	4	1.000	0.267	**0.003**

The results of non-metric multi – dimensional scaling (Figure 29) reveals a clear difference between the reef and control areas based on the Bray-Curtis dissimilarity measure from a plot of the raw data (Table 20). These data highlight the presence of different biological communities in reef areas and control areas.

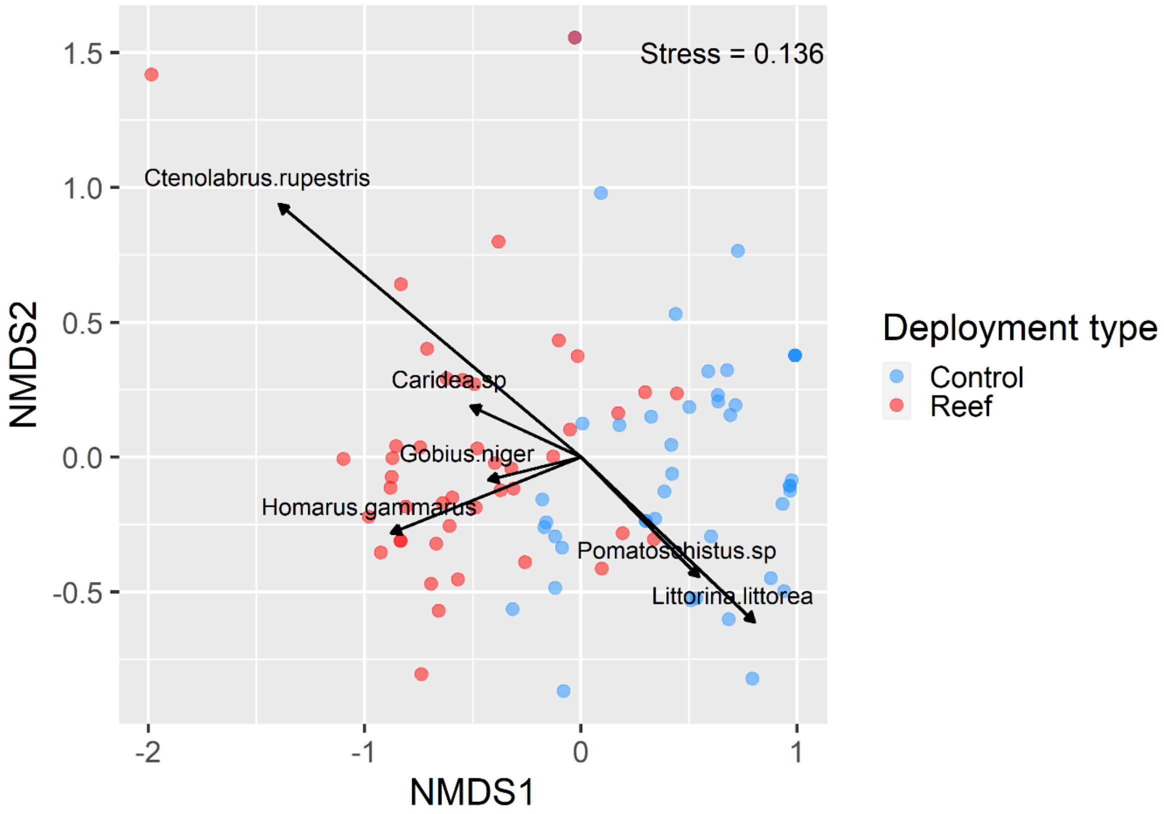


Figure 29. Results of non-metric multi-dimensional scaling (nMDS) of Bray-Curtis dissimilarity of species composition. The colours differentiate between reef areas (red) and control areas (blue). Arrows represent species significantly correlated with specific site groups from the indicator analysis (see Table 19). The length of the arrow indicates the impact of each individual species. The analysis indicates different biological communities in reef areas and control areas (Table 20).

Table 20. PERMANOVA of distance matrix using reef effect as a factor to test the difference between reef areas and control areas. The dissimilarity matrix used the Bray Curtis distances (Figure 29). The finding suggests different biological communities in reef areas and control areas.

Dependent Variable	Df	Sum of squares	Mean of squares	F value	R^2	P value
Reef effect	1	2.597	2.597	15.91	0.155	**0.001**
Residuals	88	14.203	0.163		0.845	

3.5 Discussion

The study area of Løgstør Bredning is subjected to environmental requirements by the European Union Water Framework Directive. To assess the environmental status of the Løgstør Bredning, data on chlorophyl a concentration and the maximum water depth with significant growth of eelgrass (*Zostera marina*) were downloaded from existing monitoring programs (Hansen and Høgslund, 2021). The data were plotted across time (2000-2020) and compared with environmental status thresholds developed for the European Union Water Framework Directive.

Assessment of the environmental data reveals that the environmental status deviates significantly from the requirements by the European Union Water Framework Directive. Specifically, measurements of chlorophyll a concentration (µg l^{-1}) and eelgrass maximum water depths (i.e., depth limit; m) since 2000 indicate a poor environmental status (Figure 30). Despite the degraded environment, underwater cameras deployed in the present study revealed significant effects of the artificial reefs situated in the Løgstør Bredning. Specifically, the camera data indicated reef associated biological communities and elevated biodiversity in the reef areas. The findings suggest that habitat conservation and restoration may yield positive biodiversity outcomes even in degraded aquatic environments.

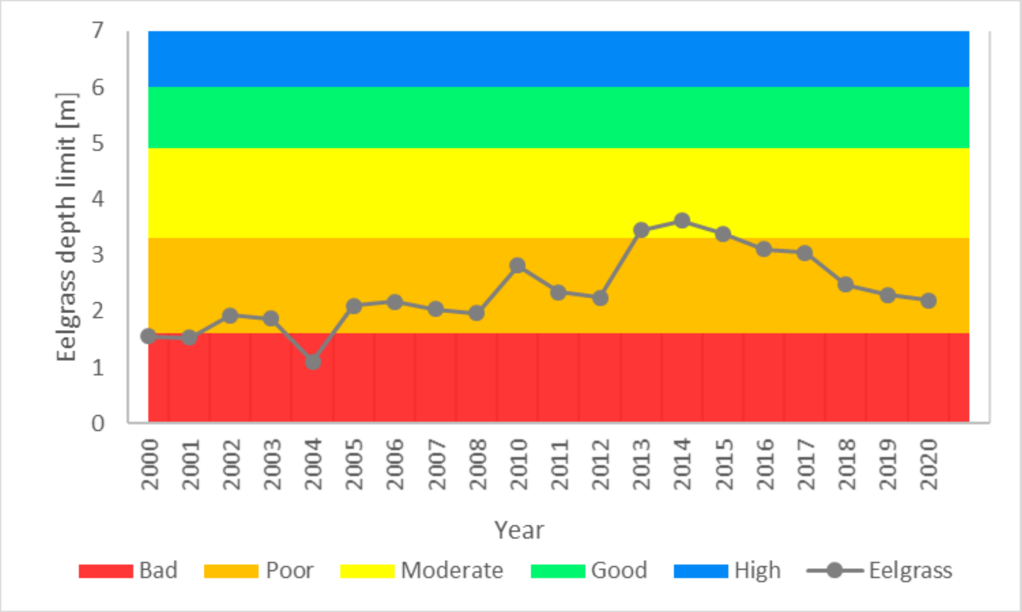


Figure 30. Annual values of the ecological indicators' chlorophyll a concentration (upper diagram; µg l^{-1}) and eelgrass depth limit (maximum water depth of occurrence; m) (lower diagram) in Løgstør Bredning in the Limfjorden in northern Denmark. Data were collected as part of the NOVANA program across the period 2000 – 2020 during the summer period (May – September). The colors represent the ecological status classes of the European Union Water Framework Directive. The directive requires the ecological indicators to demonstrate good ecological status (i.e., the green color), as a minimum. In recent years, both ecological indicators have indicated a poor environmental status for the Løgstør Bredning.

By analysing underwater video footage from reef areas and control areas in the Løgstør Bredning, this study identified significant reef effects on species abundance and marine biodiversity. Abundance estimates were based on MaxN measures derived using 60 min recordings. Data from reef areas and control areas revealed significant differences for ecologically and economically important species such as Caridean shrimp, shore crab and European lobster. The average ratio between $MaxN_{reef}$ and $MaxN_{control}$ for the European lobster was 6.5, indicating considerably higher abundances of European lobster in the reef areas. In contrast, the average ratio between $MaxN_{reef}$ and $MaxN_{control}$ for shore crab was 0.5:1, indicating lower abundances of shore crab in the reef areas. All three diversity indices assessed in this study (i.e., species richness, Shannon-Wiener index, and Pielou's Evenness index) were significantly higher in the reef areas, indicating that the artificial reefs hosted elevated local biodiversity. In addition, analyses of the biological communities indicated significant differences in species composition between reefs and control areas, driven by species such as European lobster and goldsinny wrasse. Our study shows that artificial reefs may induce significant biological changes, despite being in severely degraded marine environments. Thus, habitat modifications may change biological communities, biodiversity measures and species abundances, even if the local environment remains degraded by eutrophication and other anthropogenic disturbances.

Recent studies have shown that various fish species and European lobster benefit directly from marine protected areas (MPAs), and increased fish and lobster population densities, survivals and body sizes are often recorded inside MPAs (Kleiven et al., 2019; Moland et al., 2021; Moland et al., 2013a; Moland et al., 2013b). The reefs examined in the present study were all located inside a MPA near the island Livø in the Limfjorden. In total, 45 BRUVS were deployed on the reefs to estimate animal abundances and biodiversity using underwater footage and the resulting measures of MaxN. Because the reefs are located inside an MPA, control areas also situated inside MPAs were required to detect the habitat effects of the reefs, isolated from the MPA effects. To this end, the present study used control areas located inside three different MPAs approximately 0.2-8 km from the examined reefs. This approach isolated the effects of the reef habitats and ensured that the results are not confounded by MPA effects varying spatially. On the other hand, further research is needed to clarify if corresponding biological reef effects are present, if reef areas and control areas are compared outside of MPAs where ongoing fishing activities may change abundance patterns and biodiversity. In support of the present study, however, recent research has demonstrated that several species are responding to the presence of rocky substrate, and rocky reef restoration, despite the areas being open for ongoing fishing activities (Kristensen et al., 2017; Rhodes et al., 2020; Støttrup et al., 2014; Wilms et al., 2021). These findings suggest that habitat restoration projects in the Limfjorden would have favourable outcomes even if conducted in unprotected areas.

This study used species indicator analysis to reveal a higher MaxN of goldsinny wrasse (*Ctenolabris rupestris*) in reef areas, consistent with similar studies examining rocky substrates (Rhodes et al., 2020; Wilms et al., 2021). The goldsinny wrasse is a small-bodied mesopredator that plays an important trophic role as a prey item for large reef predators such as Atlantic cod (Funk et al., 2021; Wennhage & Pihl, 2002). Goldsinny wrasse has the capacity to consume significant amounts of blue mussel (Christie et al., 2020) and may also indirectly shape benthic vegetation growth and distribution via predation on mesograzers such as amphipods and isopods (Östman et al., 2016). Historically, goldsinny wrasse represented little or no economic value (Sayer et al., 1993), however, the species is increasingly exploited to serve as a cleaner fish that counteracts parasite infestations in salmon aquaculture industries (Blanco Gonzalez & de Boer, 2017; Moland et al., 2021; Olsen et al., 2019). In Norway, fishing for goldsinny wrasse is impacting the abundance along the southern coastlines (Halvorsen et al., 2017). Goldsinny wrasse is rarely fished in Denmark, but Danish rocky reef habitats may have the potential to sustain a fishery supplying the salmon aquaculture industry with live specimens.

This study revealed elevated numbers of black goby (*Gobius niger*) in the reef areas and in control area A, which is the control area closest to the reef areas. Elevated numbers of black goby have also been observed close to hard structures such as foundations associated with offshore wind farms (Wilhelmsson et al., 2006) or reef structures where non-uniform structures provide shelter and refuge as well as foraging opportunities (Dahl et al., 2016). The relatively high abundance associated with control area A may be due to its proximity to the reef areas that hosted these small-bodied fish in higher numbers. The minimum distance between reef areas and control areas approached 0.2 km, however, a spill-over effect from reef areas to surrounding areas (including control area A) cannot be excluded. In contrast to the black goby, sand goby (*Pomatoschistus* sp.) was more frequently encountered in the control areas, except for reef area A, as also reflected in the species indicator analyses. The control areas were mainly comprised of a sandy seabed, and the sand goby often exhibits a preference for such softer sediments where the species tend to burrow in the sediment to avoid predators, unlike the larger black gobies that are often finding shelter in macro algae dominated areas (Prog & Magnhagen, 1988). In the present study, the reef areas revealed noticeably higher algae coverages compared to the control areas. Thus, the reef areas provided a more suitable habitat for the black goby, whereas control areas offered a more suitable habitat for the sand goby. In recent years, the distribution of the invasive round goby (*Neogobius melanostomus)* has expanded quickly (Behrens et al., 2017; Christoffersen et al., 2019), but the present study made no observations of the species.

Our analyses of the video footage indicated elevated abundances of Caridean shrimp associated with the reef areas. In agreement, previous studies have reported that species like the Baltic prawn occur in reduced numbers in areas with a sandy seabed (Łapińska & Szaniawska, 2006). In general, various shrimp species play significant roles in coastal ecosystems and may influence the composition of food chains. As a predator, shrimp prey on both epi-fauna and infauna, including other crustaceans such as ostracods (Oldendorf et al., 1985). Likewise, shrimp may also prey on juvenile shore crab shortly after recruitment in coastal waters off western Sweden (Pihl et al., 1984). Baltic prawn is mainly foraging in vegetated areas and may be vulnerable to predation in habitats with a sandy seabed (Łapińska & Szaniawska, 2006; Berglund & Bengtsson, 1981), perhaps explaining the association with the reef areas in the present study. In turn, shrimp species are consumed by several fish species, including various goby species, European turbot (*Scophthalmus maximus*) and Atlantic cod (*Gadus morhua*) (Norte-Campos et al., 1995; Pihl, 1982; Ellis et al., 2002; Isaksson et al., 1994). Various shrimp species are therefore important ecological components of coastal ecosystems (Penning et al., 2021; Łapińska & Szaniawska, 2006), and may be targeted by commercial fisheries (Schulte et al., 2020).

In the present study, an equal number of 17 unique species were observed in both reef and control areas. Analyses of the data revealed that species richness, as well as both the Shannon-Wiener and the Pielou's Evenness indices, were statistically significant and higher on average in the reef areas. Collectively, the indices indicate higher diversity and more even species abundances. Such differences between reef areas and control areas were also reflected in the analyses of the biological communities present in reef areas and control areas. For example, there were higher abundances of European lobsters and Caridean shrimp in the reef areas. A relatively high value of evenness usually indicates that communities are more homogenous or even (Smith et al., 1996), therefore the lower Pielou's Evenness in the control areas may partially reflect a community dominated by shore crab, whereas the reef areas hosted a more even community with species such as lobster, black goby, and shrimp species more consistently abundant.

Previous studies have shown that European lobsters may colonize reefs shortly after reef deployments, and rocky cavities are often the preferred habitat for the species (Smith et al., 1998). Lobsters are normally nocturnal and find shelter in reefs during the day to avoid predators and protect their exoskeleton from algal fouling. European lobster often exhibits increased activity at night, because most of their feeding activity happens during this time (Smith et al., 1998). Despite this dominating nocturnal activity, the present study revealed abundance differences between reef areas and control areas using daytime underwater recordings. Although the abundances of European lobster across the 24-hour period remains unknown, it is possible that elevated abundances would also be revealed by nocturnal recordings. Consistent with the present study, the parallel study using pot deployments reported elevated catches of European lobster in the reef areas, highlighting the importance of reef habitats for European lobster. Juvenile lobsters often feed within burrows and cavities as a means of protection from predators, while adult lobsters may also capture prey passing outside of rock cavities (Smith et al., 1998). Therefore, higher densities of European lobster may be present in the reef areas, although not detected by the underwater cameras, if the juvenile animals were sedentary within burrows and cavities.

The significant abundance of shore crab uncovered by the video analyses, and encountered in 88 of the 90 BRUVS deployments, is also reflected in previous studies, reporting Limfjorden communities dominated by crustaceans and pelagic species, including small-bodied fish such as black goby as well as jellyfish (Tomczak et al., 2013 Riisgård et al., 2012). Shore crabs often adapt to different substrates, including sand, mud and rock, and are generally opportunistic feeders, with prey selection including blue mussels (Christie et al., 2020) and small fish, dependent on prey item availability in the local area (Cohen et al., 1995). On average, the present study observed half the shore crab abundance in the reef areas as compared to the abundance in the control areas. An explanation for the lower abundance of shore crabs in the reef areas could be competition from other predators, such as European lobster, perhaps dominating the reef areas. Specifically, lobsters may prey directly on shore crab (Cooper et al., 1980). In the present study, it was apparent during the video analyses that shore crab quickly scuttled away when a lobster entered the camera field of view or was feeding on the bait. Thus, lobster may have both consumptive and non-consumptive effects on smaller species like shore crab. This is consistent with the higher Pielou's Evenness in the reef areas, and reef communities more even and less dominated by shore crab.

3.6 Perspective: a preliminary assessment of the historic fish abundance and fishing

Historically, there were significant commercial fisheries in the Limfjorden. For each of the species plaice, European eel and Atlantic cod, annual landings exceeding 1,000 tons peaked before 1920 and gradually declined into a final collapse in the early 1990s (Hoffmann, 2005; Jacobsen, 2003; Riisgård et al., 2012). The simultaneous declines were presumably caused by several factors, including the eelgrass wasting disease in the 1930s, chemical and organic pollution, oxygen depletion, overfishing, beach nourishment as well as land reclamation of shallow-water habitats and changing thermal patterns (Godet et al., 2008; Hoffmann, 2005; Jacobsen, 2003; Tomczak et al., 2013; Riisgård et al., 2012). It is important to note that overfishing is not only relevant within the Limfjorden, but also in the surrounding parts of the North Sea, because of an unquantified inflow of fish eggs and larvae from the North Sea to the Limfjorden. An ongoing citizen science project has been monitoring fish abundance in the Limfjorden since the early 2000s. The resulting data reveal no evidence of recovering fish populations (Støttrup et al., 2020). In fact, fish abundance in the Limfjorden remains dominated by small species including various sticklebacks (*Gasterosteidae* spp.) and gobies (*Gobiidae* spp.) (Støttrup et al., 2020). The abundance of European lobster started increasing in the early 2000s, and the species is now dominating catches in many areas (Støttrup et al., 2020). To begin restoring historic fisheries in the Limfjorden, we propose several factors that probably need to be addressed, including 1) elimination of severe oxygen depletion, 2) adjusting and regulating fishing methods that directly harm the environment, 3) regulation of severe avian predation on juvenile fishes, 4) establishment of no-take areas in conjunction with restoration of historic eelgrass coverage as well as geogenic and biogenic reef areas, and 5) assessment of the connectivity and inflow of juveniles (mainly fish eggs and larvae) from surrounding marine areas (e.g. the North Sea) and the ongoing fishing in those areas. We note that the five factors above are based on a preliminary assessment and should be taken with care. Future studies are needed to improve our understanding on the contribution of each specific factor and the associated management improvements for the Limfjorden.

3.7 Conclusion

Based on underwater video footage from reef areas and control areas, this study revealed several interesting findings. Data uncovered significant differences in biological communities between reef and control areas. The data are further reflected by elevated species richness, Shannon-Wiener and Pielou's Evenness biodiversity indices in the reef areas. Several fish and invertebrate species often differed in abundance between reef and control area. This includes elevated abundances of goldsinny wrasse, black goby, caridean shrimp and European lobster in the reef areas, and elevated abundances of shore crab and sand goby in the control areas. The higher abundance of the commercially important European lobster in the reef areas, as well as the presence of species with important functions in the food chain such as wrasse, shrimp, and black gobies, highlight the value of artificial reef structures as a means of improving biodiversity. However, artificial reef structures alone cannot recover historic fisheries. To this end, a wide range of changes are presumably needed, including reductions in oxygen depletion, commercial fishing, avian predation and establishment of no-take areas, historic eelgrass coverage and geogenic and biogenic reefs areas, as well as increasing the inflow of juvenile fishes from surrounding marine areas (e.g., the North Sea). Further studies are needed to 1) entangle the mechanisms underpinning the decline in fisheries for commercially attractive fish species and 2) select the most suitable approaches to restore the historic fishing in the Limfjorden.

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