Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Silje Marie Leiknes

The effect of Marine Protected Areas on the diet of Lillesand European lobster (*Homarus gammarus*): A DNA-based diet analysis approach

Master's thesis in Ocean Resources
Supervisor: Glenn Dunshea
Co-supervisor: Torkild Bakken
May 2023

NTNU
Norwegian University of
Science and Technology

Silje Marie Leiknes

The effect of Marine Protected Areas on the diet of Lillesand European lobster (*Homarus gammarus*): A DNA-based diet analysis approach

Master's thesis in Ocean Resources
Supervisor: Glenn Dunshea
Co-supervisor: Torkild Bakken
May 2023

Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

NTNU
Norwegian University of
Science and Technology

Abstract

The establishment of lobster reserves protecting the European lobster (*Homarus gammarus*) has known positive effects on lobster density and size. There is however a lack of knowledge regarding the ecology of the benthic predator and its potential community-level effects following lobster population recovery. This study provides knowledge about the composition and diversity of *H. gammarus* prey within a 'before and after control impact' (BACI) experimental design. DNA metabarcoding of fecal samples was used to explore possible variations in diet that could be attributed to lobster sex, size, and two years of protection from fishing. DNA metabarcoding, next generation sequencing, and the bioinformatic pipeline used in this project led to the successful detection of one hundred and one amplicon sequence variants (ASVs) identified to a wide range of likely prey taxa.

There were no observed differences in *H. gammarus* diet attributable to the lobster reserve establishment. If the *H. gammarus* diet changes in response to recovering populations inside reserves, it seems that two years of protection was not sufficient to produce such changes. Continued monitoring of the *H. gammarus* diet will likely provide additional information about lobster ecology and the ecosystem impact of lobster reserves. Sex and size did not significantly influence the diet of *H. gammarus*, suggesting different sexes and sizes of lobster has similar foraging behaviors and abilities. The most important prey groups recognized in this study were hydrozoans, scyphozoans ('true jellyfish'), fish, tunicates, echinoderms, and crustaceans. The importance of cnidarians and tunicates suggests that soft-bodied organisms have been largely underestimated in the previously assumed diet composition of *H. gammarus*. It is also likely that the prevalence of fish has been underestimated, while the importance of hard-bodied prey has been overestimated in previous lobster diet descriptions. Lillesand *H. gammarus* can be described as scavengers and generalist predators likely to obtain parts its food from detrivory. The results also suggest they feed opportunistically by consuming items mostly based on availability. This study is the first to use molecular methods to describe the diet of *H. gammarus* and to explore the community-level effects of establishing reserves protecting the lobster species.

Sammendrag

Nedgangen i den norske bestanden av Europeisk hummer (*Homarus gammarus*) har blant annet ført til etableringen av over 50 fredningsområder for hummer siden starten av 2000-tallet. Til tross for de kjente positive effektene av fredningsområdene på hummerens størrelse og antall har det hittil vært lite fokus på hummerens økologi og effekt på det bentiske økosystemet som respons på fredning. I dette prosjektet har jeg beskrevet dietten til *H. gammarus* ved hjelp av DNA-basert diettanalyse av ekskrementprøver tatt fra hummer i et nyetablert fredningsområde i Lillesand, Norge. Komposisjonen av organismer konsumert av *H. gammarus* ble sammenlignet på tvers av område (fredningsområde og kontrollområde) og tid (før og etter etableringen av fredningsområdet). Det ble også undersøkt om dietten blant individer av ulike kjønn og størrelseskategorier varierte. Ved hjelp av DNA-metastrekkoding, nestegenerasjons-sekvensering og bioinformatiske metoder ble det påvist 101 amplikonsekvensvarianter i 92 prøver identifisert til biologiske taksoner.

Fredningsområdet hadde ingen signifikant effekt på dietten til hummeren etter to år. Likevel kan langvarig overvåkning av dietten til *H. gammarus* i Lillesand potensielt avdekke diettforskjeller om de skulle oppstå. Det var ingen signifikant diettforskjell mellom individer av ulike kjønn eller størrelseskategorier. Dette indikerer at byttedyrpreferanse og strategi brukt i søken etter mat er uavhengig av *H. gammarus* kjønn og størrelse, i det minste i den aktuelle populasjonen. De viktigste komponentene i dietten til *H. gammarus* var nesledyr, fisk, kappedyr, pigghuder, og krepsdyr. Nesledyr, kappedyr og fisk ble funnet i store relative mengder i mesteparten av prøvene, som indikerer at tidligere beskrivelse av dietten til *H. gammarus* har undervurdert viktigheten av myke organismer og fisk. I tillegg har viktigheten av organismer med harde ytre skall som bløtdyr og store krepsdyr tilsynelatende blitt overvurdert. Basert på resultatene kan *H. gammarus* beskrives som predatorer, åtseletere, detrivorer og opportunister som konsumerer mat delvis avhengig av tilgjengelighet. Denne studien er den første til å bruke DNA-baserte metoder for å beskrive dietten til *H. gammarus* og evaluere den økologiske effekten av etableringen av et fredningsområde for arten.

Acknowledgements

I would like to express my sincerest gratitude to my supervisor Glenn Dunshea for the time and effort he has invested in guiding me throughout the whole process including field sampling, laboratory work, data processing, statistical analysis and writing. I am extremely grateful for the opportunity to work on such an interesting project and for getting the chance to acquire new skills, knowledge, and a keen interest in molecular ecology and marine conservation. I will also extend my gratitude to my co-supervisor Torkild Bakken for providing advice and support from start to finish, and for helpful feedback in the writing phase.

I also want to thank Tord Amundsen at Green Bay AS, and his fantastic crew for helping us obtain fecal samples and data; the Institute of Marine Research for providing field access and equipment; and Portia Joy Nillos-Kleiven for helping in the field.

I want to thank my fellow master students for the ups and downs we have shared these past two years. Lastly, I would like to thank my parents Marit and Bjørn for their constant support during my studies in Trondheim; my sisters Amalie and Sandra for reminding me to take breaks and find room for fun during stressful periods; and Eirik for listening to both my frustrations and excitement about the project and for always encouraging me to challenge myself.

Silje Marie Leiknes,
Trondheim, May 2023

Table of Contents

1. Introduction

1.1 Background

Biodiversity loss is one of the most pressing issues leading to a reduction in ecosystem goods and services provided to human societies (Worm et al., 2006). One of the greatest threats to biodiversity is unsustainable extractive activities targeting one or a few species (Jaureguiberry et al., 2022), which disrupts ecosystem structure and thus function (Estes & Palmisano, 1974; McCauley et al., 2010; Myers et al., 2007). Marine ecosystems are especially affected by intensive fishing regimes and the habitat destruction which is a result of technological advancements to extractive gear, methods and vessels (Jackson et al., 2001; Kleiven et al., 2022; Marchal et al., 2007). Efforts to mitigate these destructive effects include the introduction of conservation management, which in later years has shifted from a single-species focus to an ecosystem-based management that recognizes the importance of protecting biodiversity for maintaining ecosystem integrity (Christensen et al., 1996).

Marine protected areas (MPAs) are sections of the oceans protected from human activity with the primary objective of conserving biodiversity and ensuring sustainable stock sizes for fisheries (IUCN WCPA, 2018). Benefits associated with MPA establishment are increases in size and abundance of target and non-target species inside MPAs compared to baseline data and controls (Babcock et al., 2010; Harmelin-Vivien et al., 2008; Shears et al., 2006; Williamson et al., 2004). Reduced fishing pressure leading to stronger juvenile recruitment effects and emigration out of the MPA (spillover) results in increased fishery catches in surrounding areas (Kelly et al., 2000; Rowley, 1994). Spillover can be caused by density independence when an individual's home range spans both the MPA and the outside (Lizaso et al., 2000). It can also be initiated by density dependence if an increase in abundance inside the MPA leads to increased intra-specific competitive interactions, precipitating migration to areas of lower conspecific density outside of the protected area (Abesamis & Russ, 2005). In addition to spillover effects and their flow-on fishery benefits, MPAs also offer varying ecological and evolutionary effects (Lester & Halpern, 2008). Selectivity of larger body sizes by fisheries has contributed to a selection of smaller and slower-growing individuals in fished populations (Biro & Sampson, 2015; Sørdalen et al., 2020, 2022). Marine protection has shown to increase genetic diversity and restore evolutionary processes leading to older, larger, and faster-growing individuals with higher fecundity (Díaz et al., 2011).

As the term MPA covers any marine area with restrictions on human use, area classifications range from lightly protected to fully protected. Fully protected MPAs prohibiting all extractive activities have been shown to be more effective than partially protected areas in restoring biodiversity and increasing fisheries success (Giakoumi et al., 2017; Sala & Giakoumi, 2018). Nevertheless, partially protected areas have been reported to offer value where full protection is not an option due to political, economic or social reasons (Sciberras et al., 2013). MPAs protecting species at higher trophic levels have for instance been very effective at causing trophic cascades restoring ecosystem complexity (Kawamata & Taino, 2021; Mumby et al., 2007). Over the past few decades, several associations such as the UN Convention on Biological Diversity (CBD) and the International Union for Conservation of Nature (IUCN) have developed detailed and ambitious goals for MPA establishment (CBD, 2021; O'Leary et al., 2016). There is an emergent need for insight into their broader effects beyond single species spillover and providing provisioning services (Leenhardt et al., 2015). For example, understanding the community ecology within MPAs is essential for filling knowledge gaps about their effects on ecosystem function and services (Cheng et al., 2019). MPA efficacy is commonly evaluated using a 'before and after control impact' (BACI) experimental design. Data is collected from the MPA and a comparable control area lacking protection before and after the MPA is established. The BACI experimental framework could be a valuable method for assessing the effect of MPAs on trophic interactions and ecosystems. In ecological impact studies, the method is known for creating statistically robust data as both spatial and temporal variability is accounted for (Sciberras et al., 2013; Smokorowski & Randall, 2017).

Since the early 2000s, over 50 partially protected MPAs prohibiting the use of gear targeting the European lobster (*Homarus gammarus*) (Linnaeus, 1758), hereafter referred to as lobster reserves, have been established in Norway (Knutsen et al., 2022). *H. gammarus* is a valuable species of high recreational and commercial interest (Franz, 2006; Kleiven et al., 2011), and analyses of catch per unit effort (CPUE) and total landings over the past half-century indicate severe overexploitation (Browne et al., 2001). In 2021 they were considered vulnerable on the Norwegian Red List due to the historic low population sizes (Tandberg et al., 2021), with an estimated 92% decrease in the Norwegian stocks of *H. gammarus* between 1928 and 2019 (Kleiven et al., 2022). Although largely based on studies of the American lobster (*H. americanus*), *H. gammarus* is assumed to be a benthic top-predator (Rozemeijer & Wolfshaar, 2019). Marine predators have the potential of affecting community structure and function through trophic cascades and inter-specific interactions (Estes & Duggins, 1995; Pinnegar et

al., 2000; Scheffer et al., 2000). A trophic cascade is caused by the removal of a species from a food web, or an increase or reduction in their biomass, resulting in indirect effects that will propagate through several trophic linkages (Pace et al., 1999). An increase in the biomass of *H. gammarus* can for instance lead to 'top-down control', where the predator regulates the lower trophic level which partly liberates the successive trophic level from predation pressure. Several long-term BACI studies of lobster reserves have contributed to important scientific knowledge of their efficacy. Yet, most studies focus only on the size, movement, and abundance of *H. gammarus* individuals (Fernández-Chacón et al., 2015; Knutsen et al., 2022; Moland, Olsen, Andvord, et al., 2011; Moland et al., 2013). No studies in Norway have yet explored the effect on interactions within communities and among trophic levels as *H. gammarus* size and abundance increases.

Examination of the diversity and composition of a predator's diet can offer insights into local biodiversity and community interactions, and explain management regime influences on these dynamics (Djurhuus et al., 2020). The prevailing method for diet analyses has been the taxonomic assignment of partially or fully digested material from the stomach contents or feces of an organism (Gosselin et al., 2017). This method is biased as most of the material is either lost or impossible to identify. Moreover, the digestion of different taxa is dependent on the prey organism's structure and composition (Alonso et al., 2014). Soft-bodied organisms are for instance processed at a higher rate and to a higher degree than arthropods and vertebrates, resulting in an under-representation of their quantity and diversity (Hawke et al., 2022; Jo et al., 2016; Sakaguchi et al., 2017). The work is also time consuming, produces small sample sizes, and requires expertise in taxonomic identification of a wide range of taxa, and is therefore not accurate nor efficient (Richardson et al., 2000; Symondson, 2002). The crushing gastric mill in the stomachs of decapod crustaceans (Huxley, 1880), makes diet analysis of this group especially challenging as their prey is nearly unrecognizable after passing the mill (Williams, 1981). This might explain why the diet of *H. gammarus* is largely undescribed (Childress & Jury, 2006). There is currently no peer-reviewed literature describing the specific diet of adult *H. gammarus*. Such information is important for understanding the species' ecology and community impact.

A DNA-based approach to diet analysis can allow for prey identification as species-specific DNA fragments persist during digestion (Dunshea, 2009). By targeting loci informative for DNA-based species identification (Taberlet et al., 2012) and employing polymerase chain

reaction (PCR) on mixed template (i.e diet) samples, DNA metabarcoding (Pompanon et al., 2011; Riaz et al., 2011) can be used for identification of numerous species in a single mixed-template sample. DNA metabarcoding has been proven a timesaving, cost-effective and accurate method of DNA-based diet analysis for invertebrate predators (Bonato et al., 2021; Miller-ter Kuile et al., 2022). It has been used to characterize food web dynamics based on the diet of species with various ecological functions (Batuecas et al., 2022; Casey et al., 2019; N. Hardy et al., 2017; Zamora-Terol et al., 2020). The method has been successful in studies using fecal samples (Deagle et al., 2010; Dunshea et al., 2013; Pompanon et al., 2012), also in relation to assessing the effects on conservation measures (Kowalczyk et al., 2011). Feces can be obtained from a predator in the field without euthanizing or otherwise harming the organism (Dunshea, 2009). DNA metabarcoding is therefore a valuable tool for evaluating the effect of lobster reserves on the lobster diet, where lethal sampling is undesirable. It should be noted that PCR of mixed template samples can introduce some unwanted sequence artifacts that artificially produce fragments such as primer dimers and chimerical molecules (Acinas et al., 2005; Bradley & Hillis, 1997; Brakenhoff et al., 1991). Several adjustments to metabarcoding workflow stages have been proposed to reduce and remove these artifacts (Taberlet et al., 2018), some of which were used in this project.

Because the majority of DNA present in the feces belongs to the predator itself (Deagle et al., 2006), prey DNA may be difficult to detect unless predator DNA is prevented from amplification. The use of blocking primers is a useful technique developed for enhancing the detection of rare species (De Barba et al., 2014; Deagle et al., 2009; Vestheim & Jarman, 2008). Blocking primers are DNA oligonucleotides with a modified non-extendable 3’ end to which the DNA polymerase in PCRs cannot bind to and extend from. They are designed to anneal specifically around the same site as the regular metabarcoding primers to out-compete them in annealing to predator DNA, subsequently blocking amplification of predator DNA. Blocking primers have been proven successful in excluding predator DNA in DNA-based dietary studies (Shehzad et al., 2012). The method allows for the use of general primer sets for covering a wide range of taxa without amplifying large quantities of predator DNA (Vestheim & Jarman, 2008). Figure 1.1 is a visual representation of PCR using blocking primers to block amplification of predator DNA.

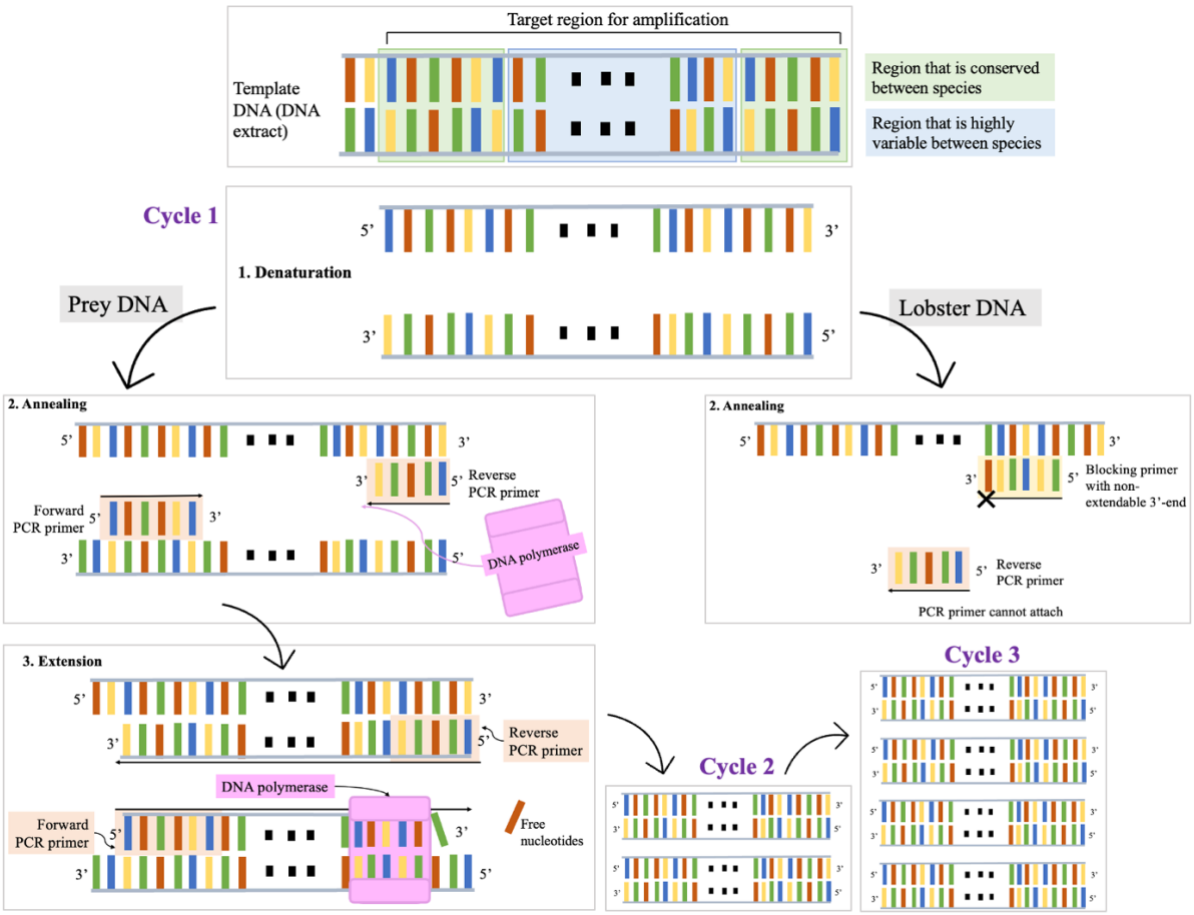

Figure 1.1: Principles behind the Polymerase Chain Reaction (PCR) using blocking primers. Both lobster and prey DNA is denatured in the first step as the temperature increases. In step 2 (Annealing), blocking primers will attach to lobster DNA, blocking the metabarcoding PCR primers from annealing and the DNA polymerase from extending. As blocking primers do not match prey DNA sequences, PCR primers anneal to the primer binding sites unhindered. In step 3, the DNA polymerase enzyme will attach to the 3'-end of the PCR primers and add free nucleotides to the growing DNA molecule until the next cycle initiates another denaturation. This will continue to be repeated until the predetermined number of cycles are completed. In every cycle, the amount of PCR product doubles (i.e., exponential amplification). Thermocycling is followed by a final 10-minute extension time (Taberlet et al, 2018) with subsequent cooling until PCR product is collected from the thermocycler. Illustration: Silje Marie Leiknes

The choice of PCR primers and target regions is a crucial step in DNA metabarcoding. DNA fragments in fecal samples are usually shorter and of lower quality than the DNA in undigested material because of the partial degradation of DNA during digestion (Deagle et al., 2006; Frantzen et al., 1998; Symondson, 2002; Troedsson et al., 2009). Using primers targeting short markers have been shown to increase amplification success and limit the production of artifacts

(Deagle et al. 2006). Primers that provide high taxonomic coverage can be designed for some markers if the marker contains highly conserved areas that many species have in common (Jusino et al., 2019; Valentini et al., 2009). Markers with a lot of within-species similarities and between-species variation provide high taxonomic resolution, enabling precise taxonomic assignment. A shorter version of the mitochondrial cytochrome c oxidase subunit I (COI) barcode has a high taxonomic resolution such that species-specific blocking primers can be designed for the marker. The COI gene also has a large and well-developed reference library. Yet, due to the high variability, the marker provides a lower taxonomic coverage where taxonomic groups can be neglected (Yu et al., 2012). The use of additional markers has been proven effective in recovering a wider set of species (Alberdi et al., 2018; Moszczynska et al., 2009). An example of a suitable additional marker is the highly conserved 18S ribosomal RNA marker (18S), for which one can design 'universal primers' capturing essentially all taxa (Taberlet et al., 2018). However, due to its low between-species variability, predator-specific blocking primers cannot be designed for this marker without also blocking phylogenetically similar taxa to the predator itself.

In this study, a DNA metabarcoding approach was employed to assess the diet of the *H. gammarus* for the first time. The obtained diet information was then used to explore potential differences in the diet of *H. gammarus* in protected (lobster reserve) and unprotected (control area) habitats, and also facilitated examining demographic variation in diet. In October 2020, a lobster reserve was established in an area near Lillesand, Norway, prohibiting lobster fishing. In August 2020, two months before implementation of the reserve, 111 fecal samples and demographic data from *H. gammarus* were collected in the to-be-designated reserve and a nearby control area by Glenn Dunshea (NTNU University Museum). In August 2022, roughly two years after lobster reserve establishment, these areas were resampled as part of the local monitoring program. Thus, samples and data collected in 2020 and 2022 provide the information needed for the BACI study design exploring differences in *H. gammarus* diet composition before and after lobster reserve implementation.

1.2 Objectives and hypotheses

The objectives of this study are to:

1) Evaluate if potential differences in Lillesand *Homarus gammarus* diet between the reserve and spatial or temporal controls may be attributed to lobster reserve establishment. This information will contribute to an increased understanding of lobster reserve effects on the Norwegian coastal benthic communities as variations in composition and diversity of lobster prey might reflect community dynamics.
2) Assess demographic variation in *H. gammarus* diet to provide further understanding of lobster ecology. Lobster size and sex might influence their prey preference and ability to catch and consume prey. And, as lobster size has been demonstrated to increase in protected areas, it is valuable to explore how this change in population size structure affects the surrounding community.
3) Produce a previously unrealized and thorough description of *H. gammarus* diet, given current information is largely anecdotal or based on studies of other lobster species employing traditional stomach contents analyses. The use of DNA metabarcoding and HTS methods might reveal unexpected diet composition as this method is less-biased than hard-part analysis (Paula & Andow, 2023) and has not been applied to *H. gammarus* or its close relatives.

To reach these objectives, the following research questions will be explored:

1) Did the establishment of the Lillesand lobster reserve and subsequent change in lobster density and population structure contribute to an observable difference in *H. gammarus* diet in the lobster reserve two years after implementation?
2) Is there a discernable distinction between the diet of *H. gammarus* belonging to different sexes and size classes?
3) What is the taxonomic composition of the *H. gammarus* diet, and what is the relative prevalence of soft-bodied organisms?

2. Materials and methods

2.1 Study area

The Skallefjord and part of Blindeia lobster reserve (1.75 km^2) and a nearby control area (Figure 2.1) in Lillesand municipality make up the study site. The Directorate of Fisheries established the reserve as a conservation zone for *H. gammarus* after regulation J-150-2020 on October 1st, 2020. The regulation forbids lobster fishing by only allowing the use of hook and line. The Directorate of Fisheries, the Coast Guard and local police are responsible for enforcing these regulations. The lobster reserve and control site are adjacent and have the same approximate size and similar habitat types with a mix of sandy and rocky bottom substrate. They are both areas assumed appropriate for lobsters. Before reserve establishment in 2020, the area was cleaned for ghost fishing gear by Green-Bay AS.

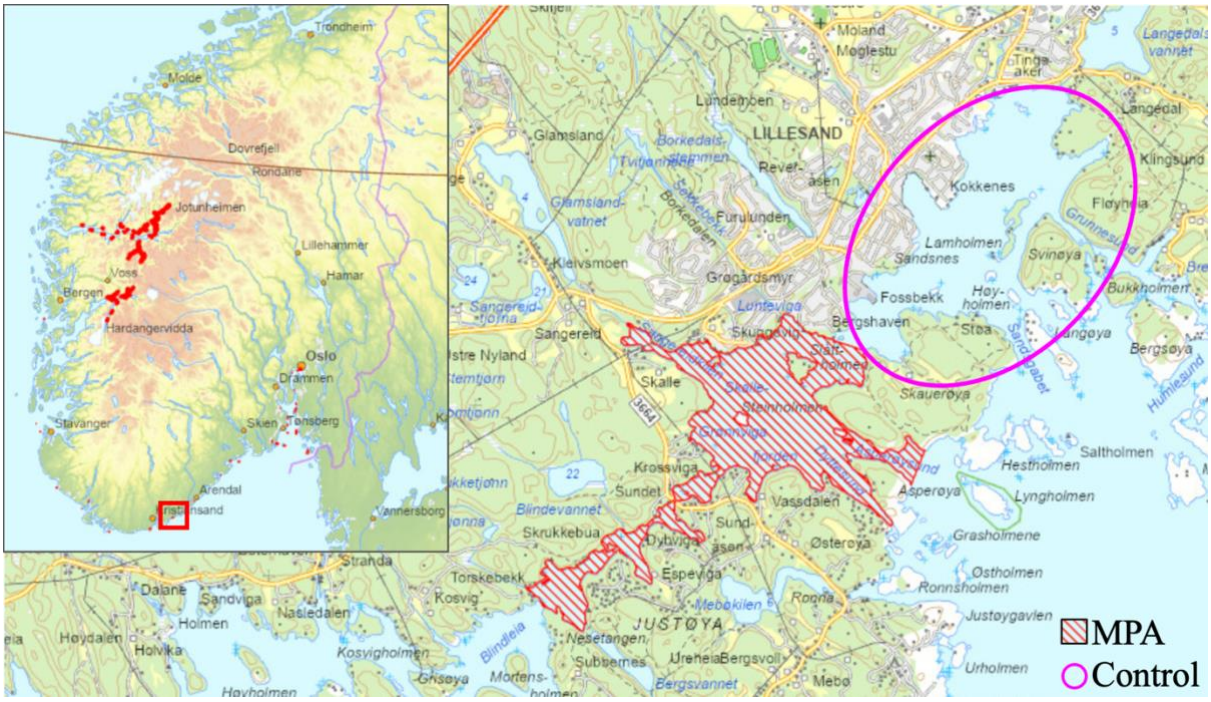

Figure 2.1: Overview of the locality of the Lillesand reserve (MPA, red striped area) and control site (Control, approximate area within the magenta colored ring). Source: Plan og Sjøareal, Fiskeridirektoratet.

2.2 Data collection

In the data collection in 2020, lobsters were obtained by deploying 25 traps, at 10-30 meters depth, in each of the two areas on august 16th. The traps were baited with mackerel (*Scomber scombrus*). Following overnight soaking, all traps were checked and moved to new locations each day for four consecutive days (n = 100 per site) from August 17th to august 20th. Most traps were a standard size for lobster fishing, except for the five smaller and five larger traps

that were deployed in each of the two areas with the intention of capturing lobsters from a wide range of sizes. The traps were placed in areas that were assumed appropriate habitats for lobster. Lobster size was measured as the total length (TL, tip of rostrum to posterior margin of telson) and the carapace length (CL, from the rear of the eye socket to the posterior edge of the carapace) (Figure 2.2). Sex was determined as female (F), female rearing (FR) or male (M). The size and sex of bycatch species was also measured and determined in the field.

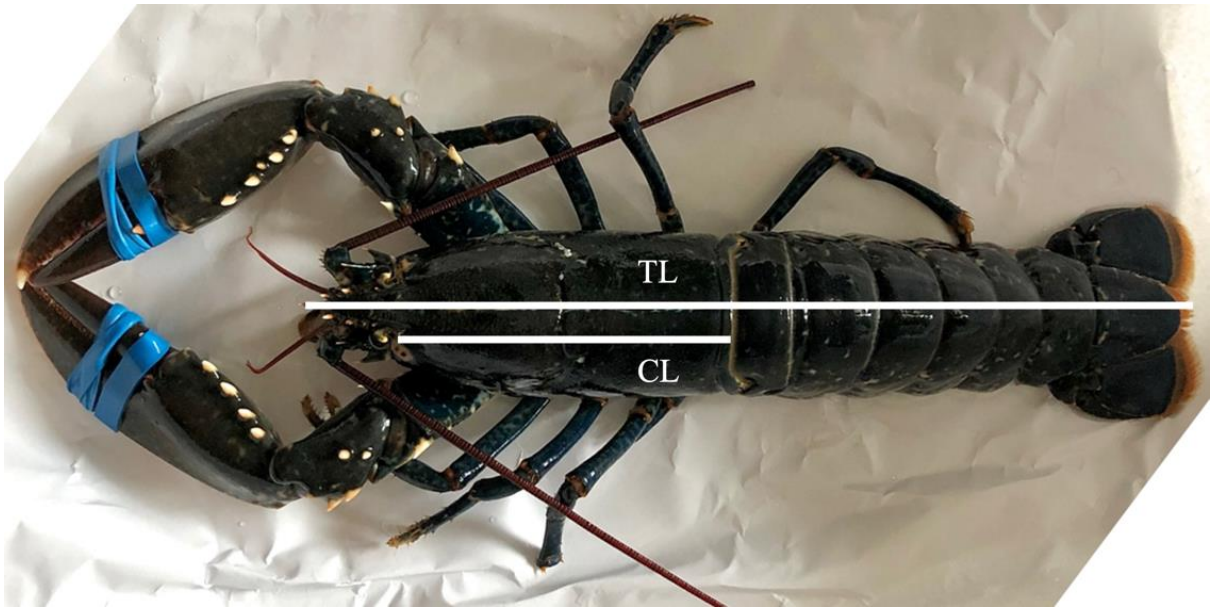

Figure 2.2: Total length (TL) and carapace length (CL) of trapped lobster was measured as shown. Photo: Silje Marie Leiknes

Fecal samples were collected non-lethally by restraining the lobster to a board with straps and inserting a pipette tip (1 ml) into the anal cavity before gently aspirating and transferring the resultant fecal sample to a microcentrifuge tube containing ethanol (96%) for preservation (Figure 2.3) (Redd et al., 2014). The filtered pipette tips were sterile and single use to avoid cross contamination between samples. After data collection and sampling, lobsters were carefully released at their respective capture sites. Fecal samples and data were obtained in collaboration with Green-Bay AS. The Institute of Marine Research (IMR) provided field access and resources. Experimental fishing was done under IMRs lobster monitoring program. No permits were required for collection of fecal samples from *H. gammarus*.

An internal control sample was collected in the field by inserting a pipette tip into the anal cavity of a lobster without aspirating fecal material. An external control was collected by sweeping a pipette tip over the anal pore. Tips were submerged into new, separate

microcentrifuge tubes containing ethanol that was pipetted a few times until the tips were removed and discarded.

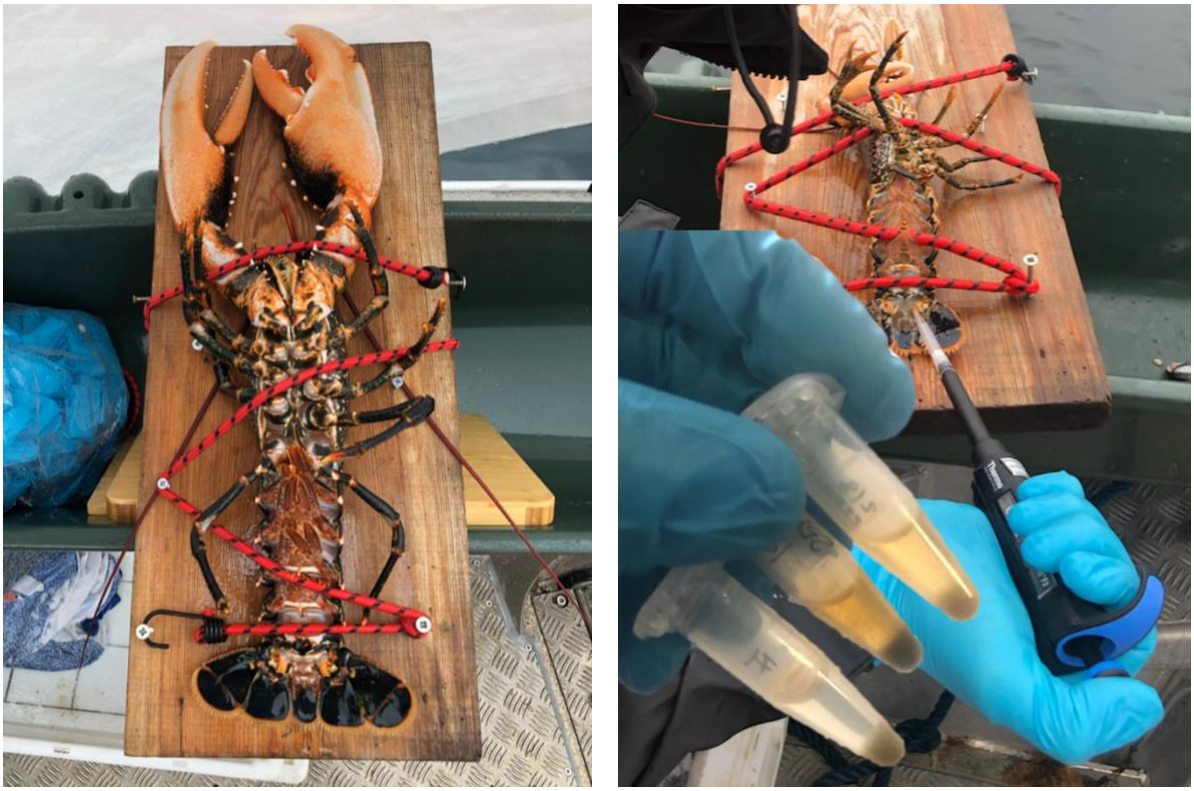

Figure 2.3: Lobster restraining method for fecal sample extraction (left). Non-destructive fecal sampling of free-ranging *H. gammarus* and resulting fecal samples preserved in ethanol (right). Photos: Silje Marie Leiknes

Data collection was executed correspondingly in 2022, apart from using 30 traps in each area (n=120 per site) and conducting fishing from August 2nd to August 5th.

2.3 Lab methods

2.3.1 PCR primer selection

The mitochondrial cytochrome c oxidase subunit I gene (COI) and eukaryotic 18S ribosomal RNA (18S) were used in combination to improve the detection of prey taxa (Alberdi et al., 2018). Primer sets for the COI gene were tested on DNA extracted from tissue samples of *H. gammarus*, Canadian lobster (*H. americanus*), blue mussel (*Mytilus edulis*), shrimp (*Pandalus borealis*) and norwegian lobster (*Nephrops norvegicus*) to aid in designing of the blocking primer for the COI locus (see Appendix B). 'Universal' (for all eukaryotes) 18S primers were tested on DNA extractions of fecal samples to confirm successful amplification, these primers

were also ultimately used for metabarcoding of sequences (see Section 3.3). PCR primers used in the initial PCR in this study (synthesized by Eurofins Genomics) are presented in table 2.1.

Table 2.1: COI and 18S PCR primers used in the initial PCR and expected PCR product size in base pairs (bp). Primer sequences are read in the 5'- to 3'-end direction. *F = forward and R = reverse.

Genomic region	Primer name	Primer direction*	Primer sequence (5'-3')	Expected PCR product size (bp)	Source
COI	nsCOIFo	F	THATRATNGGNGGN TTYGGNAAHTG	124	(Günther et al., 2018)
	mlCOIintK	R	CCWTGWCCWACTT GWCAWATRGGRGG	124	(Günther et al., 2018)
18S	All18SF	F	TGGTGCATGGCCGT TCTTAGT	150	(C. M. Hardy et al., 2010)
	All18SR	R	CATCTAAGGGCATC ACAGACC	150	(C. M. Hardy et al., 2010)

2.3.2 Blocking primer design and selection

Blocking primers for the COI locus were designed using *primer3* and the primer design feature in *Geneious Prime* (Version 2022.2.2) using standard primer picking conditions. Candidate blocking primer sequences were visually checked for all species with similar target COI sequences to *H. gammarus* in *Geneious Prime* to check for nucleotide variation such that the blocking primers would only anneal to *H. gammarus* DNA and/or that other species with similar sequences were irrelevant (e.g., inhabits tropical seas or fresh water). Suitable blocking primers were tested on extracted DNA from *H. gammarus*, *H. americanus*, *M. edulis*, *P. borealis* and *N. norvegicus* using quantitative PCR (qPCR) and gel electrophoresis (2% agarose, 110V, 1 hour) to find the most effective primer combination (see Appendix B). The chosen blocking primer, which blocked *H. americanus* and *H. gammarus* DNA but amplified *N. norvegicus* had the following forward sequence: 5'CGGCAACTGACTTGTACCAC (SpC3)3'. The SpC3 on the 3'-end of the blocking primer is a C3 spacer that blocks the DNA polymerase from binding (Vestheim & Jarman, 2008).

2.3.3 PCR optimization

PCR conditions for the COI primers were optimized by experimenting with varying concentrations of GC enhancer and annealing temperatures on DNA extracted from tissue samples of *H. gammarus*, *H. americanus* and *N. norvegicus* (Appendix B).

2.3.4 DNA extraction

Field samples were centrifuged (15 000 Relative Centrifugal Force (RCF), 5 min) and ethanol was removed with a pipette. Excess ethanol evaporated from the samples on a Thermo-Shaker (5 minutes, 37 °C, 1000 RCF). Extraction of prey DNA from fecal samples was initiated using the QIAamp Fast DNA Stool Mini Kit (QIAGEN) following the protocol of the manufacturer with modifications. InhibitEx buffer was added to each sample (1 ml). Samples were shaken (Tissue Lyser II, 10 min, 23 1/s), heated (Thermo-Shaker, 10 min, 70 degrees, 1 000 RCF) and shaken again (Tissue Lyser II, 3 min, 23 1/s). Proteinase K (25 ul, from the Fast Stool Mini Kit) was added, and samples were incubated overnight at 56 °C (16-20 hours). Samples were centrifuged (20 000 RCF, 1 min) to pellet particles. Supernatant (600 ul) was pipetted to fresh microcentrifuge tubes. The remaining extraction steps were executed by the QIAcube I, following the protocol of Fast DNA Stool Mini Kit for human DNA with a modification switching out a proteinase K addition step, simply with more inhibitEX buffer, to account for the overnight Proteinase K lysis that had already occurred. Samples were randomized ensuring lobster sex, size, and protection status varied between batches, minimizing possible batch effects and any potential data loss due to downstream contamination where samples would be discarded. Due to a maximum capacity of twelve tubes per Qiacube run, each run involved extraction of eleven stool samples and one blank sample that had gone through all previous extraction steps to monitor for cross-over and reagent contamination. DNA extracts and blank samples were then stored at -20°C until further analyes.

2.3.5 Template selection

All extracted DNA samples were run in qPCR using both COI and 18S primer sets. Templates showing amplification were organized into cycle groups based on the number of cycles completed before exponential amplification plateaus. This was done to reduce amplification bias affecting amplicon pool composition (Boulanger et al., 2012). Fecal samples not showing any amplification with qPCR using COI primers were excluded from further processes.

2.3.6 PCR amplification

All PCRs were set up in a in a pre-PCR laboratory, where no amplified DNA is permitted to be generated or stored, to limit contamination risk. Samples, DNA extracts – as well as PCR products and PCR libraries in a separate post-PCR laboratory – were all handled in bleach and

UV sterilized PCR cabinets. Three and five PCR plates were set up for DNA templates amplified by COI primers and 18S primers, respectively. Plates contained wells of fecal sample DNA extracts, tag-jump monitoring blanks (no primers, no template), DNA extraction blank controls, no template controls (NTCs) and positive PCR controls (PPCs) containing DNA template from a freshwater fish (*Lota lota*) (Taberlet et al., 2018). The number of cycles each PCR plate was run corresponded to the cycle groups.

Pre-indexed primer combinations were used in PCR targeting the COI locus. Four uniquely indexed replicate PCRs were made for each sample with a reaction volume of 15 µl. The contents of the reaction mix per reaction is shown in Table 2.2. Thermocycling conditions were 95°C for 10 min, then in cycles at 95°C for 25 s, 43°C for 45 s and 72°C for 45 s, followed by 72°C for 10 min.

Table 2.2: Reaction mix for one PCR reaction using the COI primer set.

Reagent	µl
AmpliTaq Gold 360 Master Mix (Applied Biosystems)	7.5
Primer – Forward [10 µM] (Eurofins Genomics)	0.5
Primer – Reverse [10 µM] (Eurofins Genomics)	0.5
Blocking primer [10 µM] (Eurofins Genomics)	0.75
Bovine Serum Albumin (Thermo Fisher Scientific)	0.15
Molecular grade water	3.85
360 GC Enhancer (Thermo Fisher Scientific)	0.75
Template	1

Four uniquely indexed replicate 18S PCRs were made for each sample with a reaction volume of 15 µl. The contents of the 18S reaction mix with a total volume 15 µl per reaction is shown in Table 2.3. Thermocycling conditions were 95°C for 10 min, then in cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 1 min, followed by 72°C for 10 min.

Table 2.3: Reaction mix for one PCR reaction using the 18S primer set.

Reagent	µl
AmpliTaq Gold 360 Master Mix (Applied Biosystems)	7.5
Primer – Forward [10 µM] (Eurofins Genomics)	0.6
Primer – Reverse [10 µM] (Eurofins Genomics)	0.6
Bovine Serum Albumin (Thermo Fisher Scientific)	0.05625
Molecular grade water	5.24375

2.3.7 Sequencing library preparation

All COI PCRs per individual sample were pooled. 10 µl of each sample pool was further pooled per plate, producing three COI amplicon pools. COI amplicon pools were cleaned with Sera-Mag select beads (Cytiva) at 1.2:1 bead to sample ratio, following the manufacturers protocol. After cleanup, the DNA was quantified using the dsDNA HS Assay kit with a Qubit 2.0 Fluorometer (Invitrogen), following manufacturer's protocol. 5 µl product was assessed with gel electrophoresis (agarose 2%, 110 V, 1 hour) stained with SYBR Safe DNA gel stain (Invitrogen). Since the gel run indicated presence of primer-dimers, a second cleanup with a 1:1 bead ratio was done to exclude primer dimers and other PCR products smaller than 200 bp. The Tagsteady method (Carøe & Bohmann, 2020) was used to build and quantify libraries for the COI locus, except, Sera-Mag select beads (1:1 ratio) were used in the final purification step. DNA concentrations were calculated using the NEBBiocalculator (https://nebiocalculator.neb.com/#!/qPCRlibQnt, version 1.15.0).

All 18S PCRs per individual sample were pooled and cleaned with Sera-Mag select beads (Cytiva) at 1:1 bead to sample ratio, following the manufacturers protocol. The DNA was quantified with Qubit 2.0 and assessed with gel electrophoresis (2%, 110 V, 1 hour). The Adapterama method (Glenn et al., 2019) with iNext fusion primers was used to build 18S locus libraries. The second PCR of the 18S plates was prepared in the pre-PCR lab by adding 10 µl of AmpliTaq Gold (AmpliTaq Gold 360 Master Mix, Applied Biosystems) and 5 µl of iNext primers (5 mM) with sample-specific indices to new 96-well plates. This was transferred to the post-PCT lab where 5 µl of cleaned 18S replicate amplicon pools were added to corresponding wells on the new plates with master mix and primers. Plates were run in a PCR machine under the following conditions: 95°C for 10 min, then 8 cycles of 98°C for 20s, 58°C for 30 s, 72°C for 30s followed by 72°C for 10 min. 10 µl of the 2nd PCR products from each sample were then combined per plate. Libraries were cleaned with Sera-Mag select beads in 1:1 ratio. Gel electrophoresis (2%, 110 V, 1 hour) was run to ensure successful indexing. DNA concentration was quantified with Qubit 2.0.

COI and 18S libraries were sequenced with the Illumina Novaseq600 High Throughput Sequencing platform (HTS) using a paired end sequencing approach (PE150 chemistry), producing a forward and reverse read for each fragment. The Adapterama method used for indexing and creating sequencing libraries is illustrated in Figure 2.4.

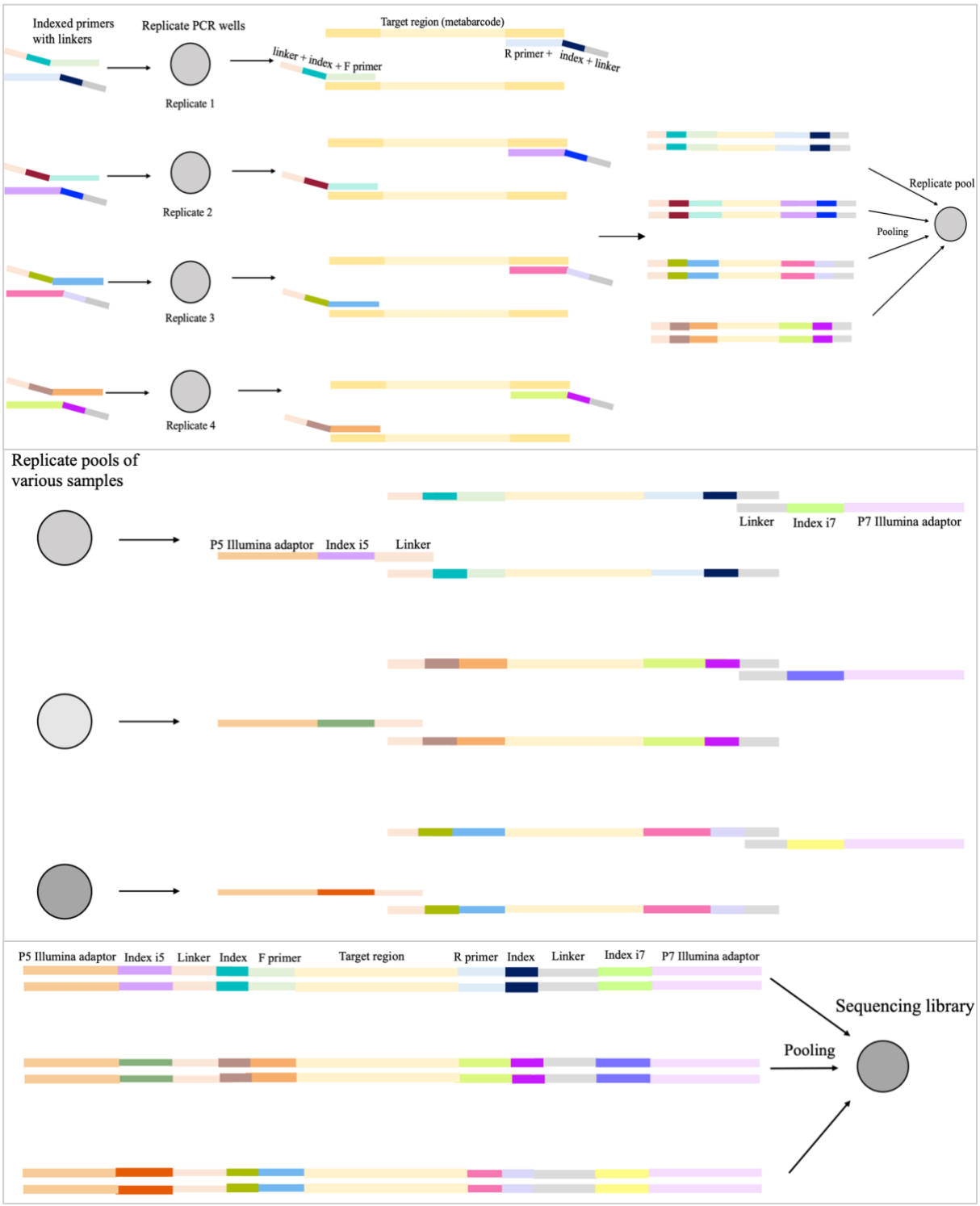

Figure 2.4: Diagram of quadruple index structure for Adapterama method using iNext primers. Top diagram: PCR primers with four different indexes amplify replicate PCRs per sample. Replicates from each sample are then pooled. Middle diagram: Pools of PCR replicates from each sample are amplified in a second PCR with primers carrying adaptors to the sequencing platform. The primers are indexed such that each sample replicate pool in a 96 well plate is individually indexed. Bottom diagram: Replicate pools of all samples in a plate are then pooled. The internal indexing differentiates between replicates, while external indexes differentiate between samples. Illustration: Silje Marie Leiknes

2.4 Data processing and analysis

2.4.1 Bioinformatics

Only the 18S locus data was processed due to failure of the COI sequencing libraries (see section 3.3). Files received from the sequencing provider consisted of demultiplexed data to the individual sample (and experimental control) level, which needed further processing to demultiplex individual PCR replicated within samples. Bioinformatic steps were executed by combining processes in the terminal (Linux) for within-sample PCR replicate demultiplexing, primer and adapter removal as well as blastn analyses. R (version 4.2.2) using the RStudio environment (version 2022.07.2) was used for sequence denoising, paired end merging, ASV table construction, curation, taxonomic data parsing and statistical analysis. R package and reference information is provided in Table 2.4. The program MEGAN6 (Metagenome Analyzer, version 6.24.20) was used for analyzing blastn results for taxonomic identification of sequences using the lowest common ancestor (LCA) approach (Huson et al., 2007). Miniconda (version 23.1.0) was installed locally and used to create a custom environment for ease of installation and access of essential programs. Using the terminal, files containing raw sequence data were placed into library (i.e. plate)-specific directories. PCR replicates in each sample were demultiplexed by finding the PCR replicate-specific forward index using *sabre* (version 1.000) (https://github.com/najoshi/sabre) (with paired-end-files and replicate-specific primer data as input), which split PCR replicates into four separate sample-specific directories and removed the sequences of forward barcodes and quality values. Since each PCR replicate had completely unique indexes (i.e. no common forward or reverse index) *Cutadapt* (version 4.3) (Martin, 2011) was used to simultaneously identify and discard PCR chimeras (unused PCR replicate index combinations) and remove primers and reverse barcodes. Trimmed files were moved to their own directory for further processing in R.

The *dada2* (Divisive Amplicon Denoising Algorithm, version 2) analysis package in R was used in filtering, sample inference, merging of paired forward and reverse reads and chimera removal. The DADA2 algorithm denoises sequences, corrects errors and produces amplicon sequence variants (ASVs) that are essentially molecular operational taxonomic units (*sensu* MOTUs or OTUs) at the 100% identity level. Using the non-pooled setting and default parameters, an ASV table was constructed consisting of all PCR replicates of all samples and controls (combined library PCR replicates of samples + controls = 1680, ASVs = 2492). Presence of chimeras was further examined using the "removeBimeraDenovo" function from

DADA2 and the ASV table was further curated using the *lulu* package, initially using BLASTn in the terminal to create a match list containing a pairwise percentage match of all ASVs in each library. The *lulu* algorithm removes spurious ASVs based on blastn sequence similarity within and between samples and the relative prevalence of ASVs within and between samples (Frøslev et al., 2017).

Following lulu curation (ASVs = 2492), all ASVs were assigned taxonomic identity by performing a BLASTn search against a local copy of the NCBI nucleotide database (Release 87, downloaded 24.02.2023), excluding unclassified/uncultured environmental sequences. BLASTn results were saved into .xml format and loaded into MEGAN 6, where the weighted LCA algorithm was applied using the following parameters, which yielded robust results given read lengths and sequence variation (Reads=2,492 Assigned=254,981 (alignedBases), MinScore=100.0, MaxExpected=0.01, MinPercentIdentity=95.0, TopPercent=5.0, MinSupportPercent=0.05, MinSupport=167, disabledTaxa=120 LCA=weighted lca, CoveragePercent=80, mode=BlastN). Contaminant ASVs were then identified and removed (n = 57 contaminants) using the *decontam* package (version 1.20.0) (Davis et al., 2018) using the "prevalence" method with the NTC, blank extract and external swab controls designated as control samples. Including the external swab control samples in this fashion accounted for potential external environmental contamination at the time of pipetting fecal samples in the field. As well as monitoring for successful amplification, PPCs in each plate were used for monitoring cross-contamination during PCR set-up, which is particularly likely to affect biological samples with low amplifiable DNA content. Here, the prevalence of the PPC ASV was examined in all 263 fecal samples and any sample with greater than a mean of 1% prevalence across sequence counts in PCR replicates, and with the PPC present in > 2 PCR replicates was considered poor quality and removed (n = 38, 14.3% of samples). The PPC out was then removed from the data matrix. For other control samples, the largest corresponding number of reads found in control samples (NTCs, no template/no primer blanks, and blank extractions) for any given batch of extractions, plate and PCR replicate was subtracted from their appropriate biological samples to control for tag jumps and background contamination. Control samples were then removed from the datasets, leaving only a biological (fecal) sample matrix consisting of individual PCR replicates from all samples. Different iterations of this matrix were then constructed where ASVs were removed based on their occurrence across PCR replicates within samples as a further quality control measure (Alberdi et al., 2017), yielding four separate sample matrices (unmodified, ASV present in 2/4 PCR replicates, 3/4 PCR

replicates, and 4/4 PCR replicates). Finally, to yield a single value per ASV per sample, sequence counts across PCR replicates were summed. A list of *Phyloseq* objects (McMurdie & Holmes, 2013) (n = 4, one per PCR replicate filtering matrix) were then constructed consisting of the ASV table (phyloseq::otu_table), ASV taxonomic designations (phyloseq::tax_table), data of samples and their characteristics (phyloseq::sample_data) and the representative sequence of each ASV (phyloseq::refseq).

Since each ASV matrices consisted of all 18S eukaryote sequences amplified, further quality control procedures were implemented to remove irrelevant ASVs (e.g. predator, microbiome and other protists, parasite etc). ASVs of predator origin were identified taxonomically by examining BLAST output (ASV 1 & ASV 18) and removed. ASVs from microorganisms and likely parasites (e.g. Platyhelminthes) were then removed so only putative forage taxa phyla were retained: The Annelida, Arthropoda, Bryozoa, Chlorophyta, Chordata, Cnidaria, Echinodermata, Haptophyta, Kinorhyncha, Mollusca, Nematoda, Nemertea, Porifera, Rhodophyta, Streptophyta. Since, as expected, in many samples the majority of sequences were of predator and microorganism origin, new phyloseq lists (of all 4 PCR replicate filtering options) were constructed that removed samples with low sequencing depth of putative forage taxa at the following thresholds: >100, >200, >500 >1000. Following sequencing depth filtering, taxa and samples with zero reads were discarded.

All code for bioinformatic routines is provided on GitHub and can be accessed with this link:https://github.com/siljeleiknes/Lobster/blob/d07aef8118ff00bed4894f00b8969236f84ebf32/Lobster_bioinf.R

2.4.2 Statistical analysis and visualization

All statistical analyses were conducted in Rstudio. Packages used for the analysis are presented in Table 2.4.

Catch per unit effort was calculated as the number of lobsters caught divided by the number of traps fished per area per day (Knutsen et al., 2022; Nillos Kleiven et al., 2019). The calculation provided an estimate of average number of lobsters caught per trap per BACI treatment group: control area 2020 (CTRL20), MPA 2020 (MPA20), control area 2022 (CTRL20), and MPA 2022 (MPA22). The averages were plotted and an Analysis of Variance (ANOVA) was

performed using the *stats* package to assess the effect of lobster reserve on lobster abundance. Model assumptions were examined with residual plots. Levene's test (using the *car* package) was performed on the size data to test for homogeneity of variance due to the skewed sample sizes. The effect of lobster reserve on lobster size was tested with Welch's ANOVA test and the Games-Howell post hoc test from the *rstatix* package. A boxplot of *H. gammarus* size per BACI group was constructed.

For each phyloseq object (PCR replicate filtered) iteration in each list (sequencing depth filtered), maximum likelihood phylogenetic trees were created using the *DECIPHER* and *phangorn* packages. For cautious interpretation and to avoid spurious inference from false positives, statistical analysis was performed on *phyloseq* objects where sequencing depth cut-off was at < 500 sequences per sample, and ASVs were retained in samples if present in 3 or 4 out of 4 PCR replicates. To illustrate the effect of replicate control, the number of taxa found in each phyloseq object at the four different replicate control thresholds was plotted.

Functions from the *phylosmith* package were used to identify common ASVs occurring in > 5% of samples and ASVs that were unique to each BACI group. Rare ASVs (present in < 2% of samples) were found using the *microbiome* package.

Sample-specific relative read abundance (RRA) was calculated to convert the sequencing reads to interpretable dietary data, as it is a recommended method for indicating the importance of diet items (Deagle et al., 2018). RRA values were averaged across samples to provide a diet composition summary with each sample weighed equally. Calculating the RRA of individual ASVs averaged by sample is done using the following formula:

$$RRA_i = \frac{1}{N} \sum_{k=1}^{N} \frac{n_{i,k}}{\sum_{i=1}^{T} n_{i,k}}$$

where N is the total number of samples, $n_{i,k}$ is the number of sequencing reads of ASV i in sample k, and T is the total number of ASVs in sample k. Sample averaged RRA (saRRA) per ASV was summed by subphylum and plotted to give an indication of overall diet composition. A phylogenetic tree (based on the maximum likelihood tree) with ASVs from the four phyla with the highest saRRA values was plotted to show ASV diversity and prevalence. An

occurrence (presence/absence of ASVs) table was constructed using the *metagMisc* package with a 0.01 detection threshold (Deagle et al., 2018). Frequency of occurrence (FOO) of ASVs present in > 5% of samples was plotted. As occurrence data artificially inflates the importance of rare taxa (Deagle et al., 2018), it was only used as a measure for the prey items with high saRRAs. saRRA was plotted against FOO to investigate the relationship between the two methods of interpreting sequencing reads.

HTS produces compositional data reflecting a sample of the relative abundances of molecules in a sample and not their absolute abundances. Therefore, log-ratio transformation is recommended for compositional data as proportions are first converted to ratios which preserves the information of the relationship between the components (Gloor et al., 2017). Applying the logarithm function to the ratios creates a coordinate space of linearly related and symmetric that can be interpreted and analyzed with multivariate statistical methods. The phylogenetic isometric log-ratio (philr) is an example of a recommended method as it also considers phylogenetic models (Silverman et al., 2017). Two PCoAs, one using total ASVs and one using common ASVs (occurring in > 5% of samples), were performed on philr transformed data using the *philr* package and plotted to explore multivariate structure in *H. gammarus* diet between the four BACI groups. A circular compositional bar plot was plotted with samples ordered by ordination (principal component analysis (PCA)) of centered log transformed (clr) data (Gloor et al., 2017). The plot was used to visually explore differences in composition among samples collected from the reserve and control area in 2020 and 2022.

Philr transformed data were analyzed with the Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001), to examine if ASV composition varied significantly between BACI groups. PERMANOVAs were run with the 'adonis2' function from the *vegan* package both testing the year (before & after) and treatment (MPA & control) sequentially with an interaction term of the two. A permutational pairwise test was done to test for differences between factor levels using the 'pairwise.adonis2' function from the package *pairwiseAdonis*. Additional PERMANOVA tests were run with the sex variable at two levels: male and female (excluding the single sample lacking information on sex), and the size variable arranged into three levels: smaller than average (1^{st} quantile), average (Mid quantile > 1^{st} quantile and < 3^{rd} quantile) and larger than average (3^{rd} quantile). Male *H. gammarus* have larger claws relative to body size compared to females, and this is especially evident within reserves (Sørdalen et al., 2020). A subset of ASVs assigned to organisms with hard exoskeletons (Echinodermata,

Mollusca, and Crustacea) was tested in PERMANOVA to see if males or larger lobsters had a higher consumption of hard organisms. All PERMANOVA tests were run with 999 permutations. A PERMIDISP test (Anderson et al., 2006) was performed with the 'betadisper' function in the *vegan* package to test for homogeneity of multivariate dispersion checking the assumptions of the PERMANOVA that all groups have an equal dispersion.

The average number and standard error of ASVs found in each sample was calculated to get a sense of diet item variation on the *H. gammarus* diet compositions. The dietary composition of individual fecal samples was plotted with *microbiome* using the RRAs of ASVs grouped by subphylum and by species to visualize the individual *H. gammarus* diets. Soft-bodied organisms, hard-bodied organisms, and organisms lacking a hard exoskeleton but having hard parts or tough tissue were divided into their own respective groups to check the overall composition of organisms likely and unlikely to be detected using traditional morphological diet analysis methods. The ASVs with highest saRRAs and FOOs believed to be important diet items for *H. gammarus* were blasted individually (NCBI, BLASTn) to explore if they could be classified further without the constraints of the MEGAN analysis conservatively assigning ASVs to higher taxonomic levels with the chosen parameters.

Table 2.4: The name, version, usage, and reference for the packages used in R for bioinformatic routines, statistical analysis, and data visualization.

Package	Version	Usage	Reference
dada2	1.26.0	Filtering, sequence denoising, paired end merging, removing chimeras, creating ASV tables	(Callahan et al., 2016)
lulu	0.1.0	Curation of ASV table	(Frøslev et al., 2017)
decontam	1.20.0	Remove contaminant ASVs	(Davis et al., 2018)
stats	3.6.2	ANOVA for CPUE	(R Core Team, 2022)
DECIPHER	2.26.0	Align sequences to make maximum likelihood phylogenetic trees	(E. S. Wright, 2016)
phylosmith	1.0.6	Identify common, unique, core taxa	(Smith, 2019)
microbiome	1.12.0	Identify rare taxa, make composition plots	(Lahti & Shetty, 2017)
philr	1.24.0	Phylogenetic isometric log-ratio transformation of data, create PCoA plots	(Silverman et al., 2017)
microViz	0.10.8	Plotting ordination	(Barnett et al., 2021)
vegan	2.6.4	PERMANOVA and PERMDISP tests	(Oksanen et al., 2022)
phyloseq	1.26.1	Creating phyloseq objects, plotting	(McMurdie & Holmes, 2013)
ggplot2	3.4.2	Plotting	(Wickham, 2016)
pairwiseAdonis	0.4.1	Permutational pairwise test	(Martinez Arbizu, 2017)

car	3.1.2	Levene's test for checking homogeinity of variance (lobster size)	(Fox & Weisberg, 2019)
pals	1.7	Color palettes	(K. Wright, 2021)
fabricatr	1.0.0	Making new variables for size	(Blair et al., 2022)
PColorBrewer	1.1-3	Color palettes	(Neuwirth, 2022)
ggsignif	0.6.4	Round numbers to significant figures	(Ahlmann-Eltze & Patil, 2021)
ggpubr	0.6.0	Plotting	(Kassambara, 2023)
phangorn	2.11.1	Make maximum likelihood phylogenetic trees from each alignment	(Schliep, 2011)
gridExtra	2.3	Grid graphics in plots	(Auguie, 2017)
taxonomizer	0.10.2	Function to work with NCBI accessions and taxonomy	(Sherrill-Mix, 2023)
taxize	0.9.100	Taxonomic information from the web	(Chamberlain & Szocs, 2013)
taxizedb	0.3.1	Tools for working with taxonomic databases	(Chamberlain & Arendsee, 2023)
BiocManager	1.30.20	Access Bioconductor project packages	(Morgan, 2023)
devtools	2.4.5	Access to packages	(Wickham et al., 2022)
seqinr	4.2-30	Retrieve and analyze biological sequences	(Charif & Lobry, 2007)
phylotools	0.2.4	Phylogentic tools	(Zhang, 2021)
dplyr	1.1.2	Manipulate data	(Wickham et al., 2023)
psadd	0.1.3	Additional functions to phyloseq package	(Pauvert, 2023)
plotrix	3.8-2	Calculate standard errors, Welch's test, Games-Howell test	(Lemon, 2006)
stringr	1.5.0	String operations for changing sample names	(Wickham, 2022)
abind	1.4-5	Combine multidimentional arrays	(Plate & Heiberger, 2016)
tidyverse	2.0.0	Set of packages	(Wickham et al., 2019)
textshape	1.7.3	Reshaping text	(Rinker, 2021)
Hmisc	5.0-1	Various functions useful for data analysis	(Harrell Jr, 2023)
data.table	1.14.8	Extension of 'data.frame'	(Dowle & Srinivasan, 2023)
metacoder	0.3.6	Plotting and manipulating taxonomic data	(Foster et al., 2017)

3. Results

3.1 Lobster catch per unit effort (CPUE)

The four days of fishing in 2020 and 2022 yielded data and fecal samples from 111 (control: 58, MPA: 53) and 152 (control: 48, MPA: 104) lobsters, respectively. The number of lobsters caught per trap (CPUE) in each BACI group is illustrated in Figure 3.1. CPUE appeared to decrease inside the control area, although not significantly, and increase in the reserve between 2020 and 2022. The two-way ANOVA revealed that there was a significant interaction between treatment and year ($F_{(3, 12)} = 5.0672$, $p = 0.044$) for lobster CPUE. No significant effect of year ($F_{(3, 12)} = 0.46$, $p = 0.51$) or treatment ($F_{(3, 12)} = 3.29$, $p = 0.095$) was found. The significance of the interaction can be explained by the considerable difference in catch success between the control area and reserve in 2022, yet no difference between areas in 2020, such that the effect of treatment is dependent on the year (Figure 3.1). Thus there was a difference in *H. gammarus* abundance, inferred from CPUE, that can be attributed to the establishment of the Lillesand lobster reserve.

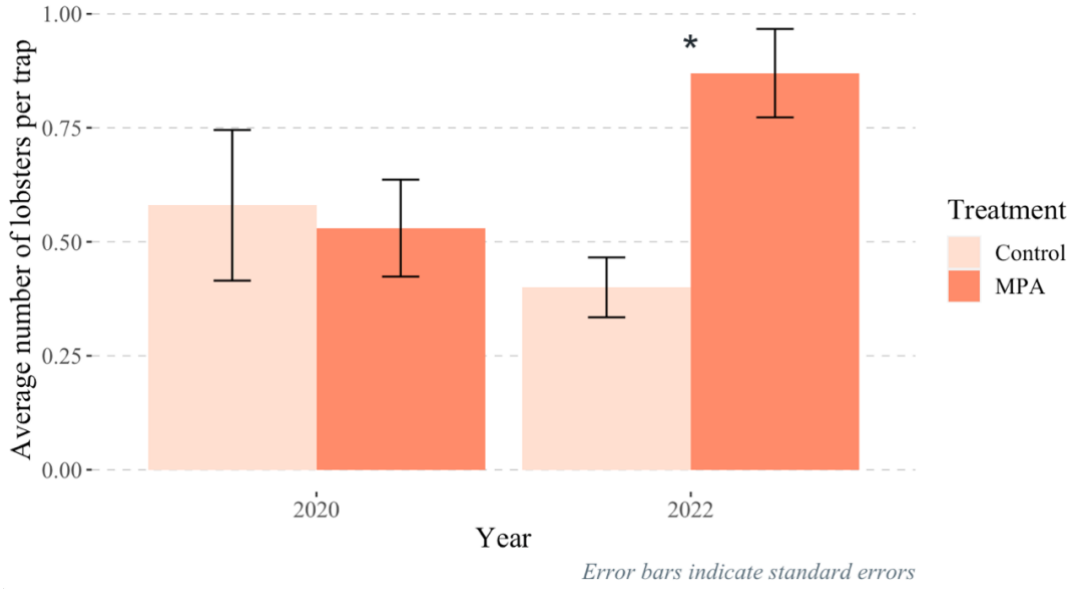

Figure 3.1: Average number of lobsters per trap in control area and MPA in 2020 and 2022 adjusted for number of traps deployed. Error bars indicate standard errors (SE), and the MPA 2022 bar is marked with an asterisk (*) to show that it is significantly different from the other groups.

3.2 Lobster size

Results from the Levene's test were significant ($F_{(3, 259)} = 4.73$, $p = 0.00315$), thus, the assumption of homogeneity of variance was not met. The Welch's ANOVA test was therefore performed on the data and gave significant results ($F_{(3, 130.06)} = 16.039$, $p = 6.25*10^{-09}$), indicating a size difference between groups. The Games-Howell post hoc test comparing all

combinations of the BACI groups showed that the only pairwise comparisons with significant results where those made between groups from different years. The median values in the boxplot in Figure 3.2 suggests that lobsters were larger in 2022. These results indicate that the size change is not attributable to reserve establishment.

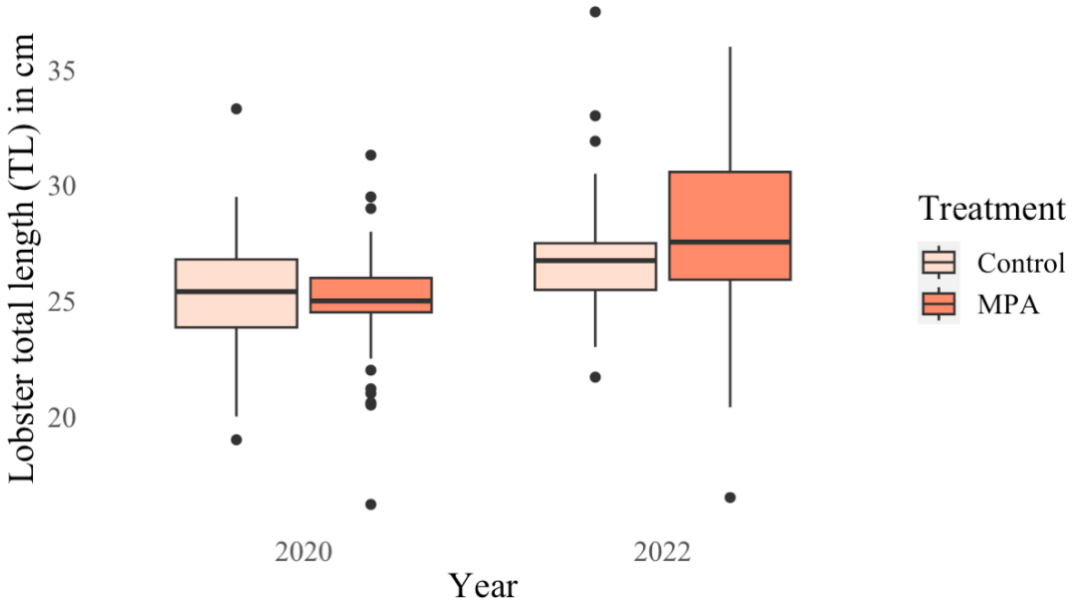

Figure 3.2: Box plot of *H. gammarus* size (TL, cm) as a function of area and year (BACI group). The boxes represent the interquartile range (IQR) between the upper (Q3) and lower (Q1) quartiles. Upper and lower whiskers indicate maximum (Q3 + 1.5 * IQR) and minimum (Q1 - 1.5 * IQR) values. Black dots represent outliers, and black horizontal lines within boxes represent the median TL.

3.3 Lab results

Gels run before constructing COI TagSteady sequencing libraries indicated marginal amplification success, but Tagsteady sequencing libraries were constructed regardless. Library quality controls performed by the sequence provider indicated that the COI libraries could not be successfully sequenced. Additional optimization experiments and bead cleaning steps (Appendix C) could not provide amplicon pools containing product of the desired size and the COI marker was thus abandoned. All subsequent results refer to the 18S rRNA primer pair only.

3.4 Data characteristics

3.4.1 Raw reads, filtering, and contamination control

445 samples & controls from 5 plates were sent to the sequencing provider (NOVOGENE, UK) for Illumina sequencing. A total of 122,742,182 raw sequence reads including fecal

samples and controls were obtained. The sum of reads per sample type and plate are listed in Table 3.1. The data quality control reports revealed high error rates and poor quality forward reads (see Appendix E). This led to substantial data loss in the initial filtering step after dereplication where 66.1%**,** 67.5%**,** 68.8%**,** 37.7%**,** 53.8% of input samples in plates 1-5, respectively, passed the filter.

Table 3.1: The sum of raw reads received from the sequencing provider (Illumina) divided by plate and sample type (fecal samples, PPCs, NTCs, tagcatches, blank extractions, field controls).

Plate	*1*	*2*	*3*	*4*	*5*
Fecal sample	24,459,610	15,907,404	22,861,058	21,117,028	20,219,340
PPC	3,006,516	2,004,886	2,457,972	1,472,560	1,742,730
NTC	42,970	565,486	29,482	187,802	842,984
Tagcatch	2,416	1,856	2,440	0	9,820
Blank extraction	-	4,363,554	-	-	632,252
Field control	-	-	-	468,706	343,310

After DADA2 quality filtering, read merging and ASV inference there was a total of 20,279,019 reads in 3539 ASVs across all plates which was reduced to 20,270,997 reads in 3539 ASVs after chimera removal. Lulu curation and removal of contaminants resulted in 2492 and 2453 ASVs, respectively. 1528 PCR replicates (including controls) remained after removing samples with an average of PPC sequences > 1% of total sequences in > 2 replicates. Some blank extraction controls in plate 2 and 5, as well as field controls in plate 4 and 5 both had high read counts (Table 3.1) and a high median per PCR replicate (Table 3.2). Sequences present in blank extractions and field controls at high abundances were either not found at meaningful abundances in fecal samples, represented DNA from taxa that were irrelevant to the *H. gammarus* diet, or both. Examples of contaminant taxa not relevant to interpretation of the *H. gammarus* diet were a few uncultured eukaryotes and chimpanzee (*Pan troglodytes*), likely from human DNA contamination in the lab. The high read counts in NTCs from plate 2 and 5 was the result of a few samples containing many sequences rarely found in other samples, except for a few unproblematic species such as *P. troglodytes*, uncultured fungus, and *Saccharomyces cerevisiae* (yeast). Since sequences from the blank extractions and NTCs were removed from samples belonging to the same extraction batch and PCR plate, respectively, and field controls did not share common taxonomic attributes with the fecal samples, biological samples are assumed to be true biological representations of the prey ASVs amplified from *H. gammarus* faeces. Removal of NTCs, blank extractions and ASVs assigned to lobster and the

positive control (*L. lota*), finally returned 1950 taxa in 900 biological PCR replicates (225 samples). As expected, most sequences were from ASVs assigned to *H. gammarus* (91.69%) and *L. lota* (0.92%). Removal of these ASVs resulted in the total loss of 92.61% of sequences.

Table 3.2: Median read numbers per PCR replicate of fecal samples and controls (field controls, blank extractions, PPCs, NTCs, tagcatches). Values represent those at various stages of the DADA2 sample inference pipeline: *input*, *filtered*, *denoised forward* and *reverse reads*, and *merged sequences*, grouped by plate.

	input	**filtered**	**denoisedF**	**denoisedR**	**merged**
Plate 1					
Fecal sample	33,041	21,836	21,820	21,822	21,805
PPC	8,505	6,871	6,867	6,871	6,866
NTC	35	20	16	20	16
Tagcatch	11	6	4	6	3
Plate 2					
Fecal sample	26,472	17,857	17,819	17,837	17,790
Blank extraction	19,226	13,069	13,029	13,032	12,826
PPC	9,622	7,164	7,157	7,162	7,157
NTC	64	42	42	42	40
Tagcatch	9	5	3	4	2
Plate 3					
Fecal sample	22,930	15,787	15,743	15,762	15,705
PPC	9,246	7,656	7,647	7,653	7,644
NTC	64	42	40	41	40
Tagcatch	9	6	5	5	4
Plate 4					
Fecal sample	21,266	8,012	7,962	7,998	7,902
Field control	37,310	9,806	9,787	9,788	9,399
PPC	4,922	2,385	2,382	2,384	2,382
NTC	0	0	0	0	0
Tagcatch	0	0	0	0	0
Plate 5					
Fecal sample	34,443	18,527	18,457	18,504	7,908
Field control	24,258	13,217	13,143	13,169	12,711
Blank extraction	15,944	8,374	8,324	8,349	8,171
PPC	7,890	4,938	4,934	4,933	4,933
NTC	139	68	63	68	62
Tagcatch	15	8	4	6	0

3.4.2 Sequencing depth and replicate filtering

There was a clear decrease in number of ASVs with an increasing replicate control threshold (Figure 3.3, Table 3.3), as well as a decrease in number of samples after removing samples with less than 500 prey reads (Table 3.3). Replicate filtering has been shown to greatly affect sequence results and decrease biases in taxonomic assignment (Alberdi et al., 2017). Of the 92 samples in the filtered dataset, 10, 9, 19, and 53 samples represented the CTRL20, MPA20, CTRL22 and MPA22, respectively.

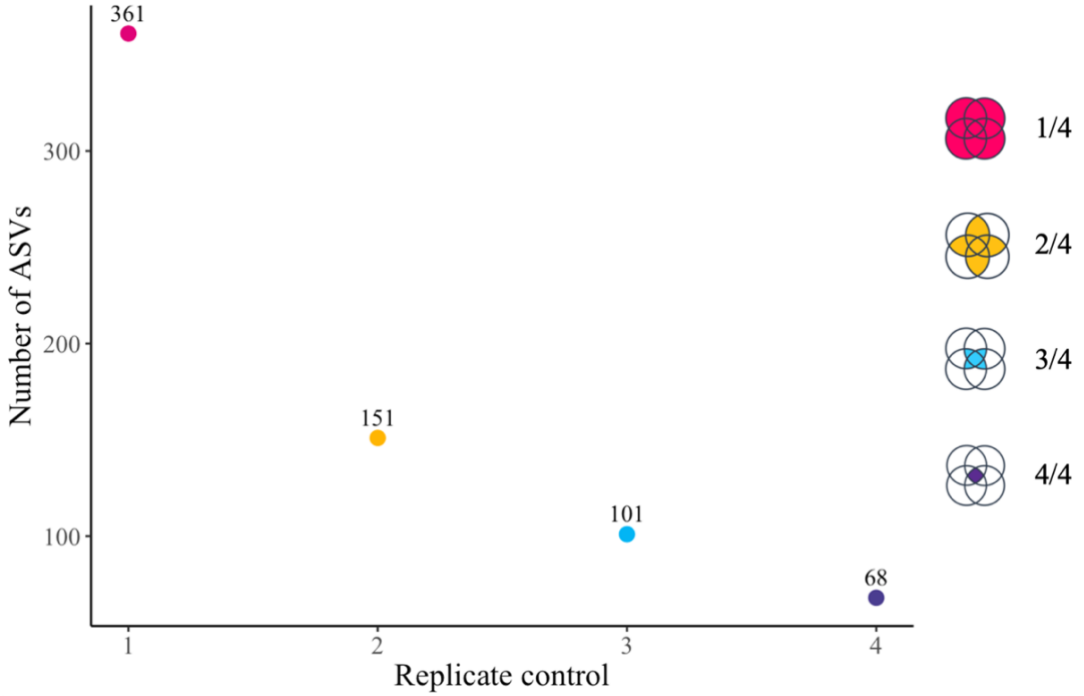

Figure 3.3: Replicate control 1, 2, 3, 4 on the x-axis represents a chosen threshold based on if an amplicon sequence variant (ASV) is present in at least 1, 2, 3 or 4 of 4 replicates per sample using a sequencing depth of > 500 diet reads. The legend diagrams illustrate the degree of restrictiveness of filtering replicate controls.

Table 3.3: Number of ASVs and samples after filtering steps using a > 500 sequencing depth threshold. *The number of ASVs and samples used in analyses (3/4 replicate threshold).

Filter	**Type**	**Replicate control**			
		1/4	***2/4***	***3/4***	***4/4***
Before filtering ASVs and samples	*ASVs*	1950	454	259	157
	Samples	225	225	225	225
Removing samples with <500 reads	*ASVs*	1950	454	259	157
	Samples	144	131	123	110
After removing non-metazoan taxa	*ASVs*	394	157	107	70
	Samples	144	131	123	110
Removing samples with <500 reads and ASVs with zero reads	*ASVs*	361	151	101*	68
	Samples	111	99	92*	83

3.5 Dietary composition

3.5.1 Taxonomic composition of fecal samples

The filtered phyloseq object consisted of 101 ASVs in 92 samples. The ASVs were identified to 14 phyla, with 31 classes, 34 orders, 29 families, 15 genera, and 7 species. Samples from CTRL20, MPA20, CTRL22, and MPA22 each contained 3, 6, 11, and 40 unique ASVs, respectively. 46 ASVs were considered rare (present in < 2% of samples), while 22 ASVs were considered common (present in > 5% of samples). See Appendix A for the full taxonomic table.

A plot of the subphylum level taxonomic composition based on ASV relative read abundance averaged across samples (saRRAs) is presented in Figure 3.4. The Cnidaria phylum was the most important prey group with a summed saRRA of 34.65%. Furthermore, the two ASVs occurring most frequently in samples (Figure 3.5) were both assigned to cnidarian taxa. ASVs assigned to Hydrozoans and Scyphozoa ('true jellyfish') within the Cnidaria phylum had summed saRRAs of 17.2%, and 15.3%, respectively. The Craniata subphylum (clade of chordates) was the second most important group when ranked by subphylum with a summed saRRA of 28.82%, followed by the Tunicata subphylum (16.16%), the Echinodermata phylum

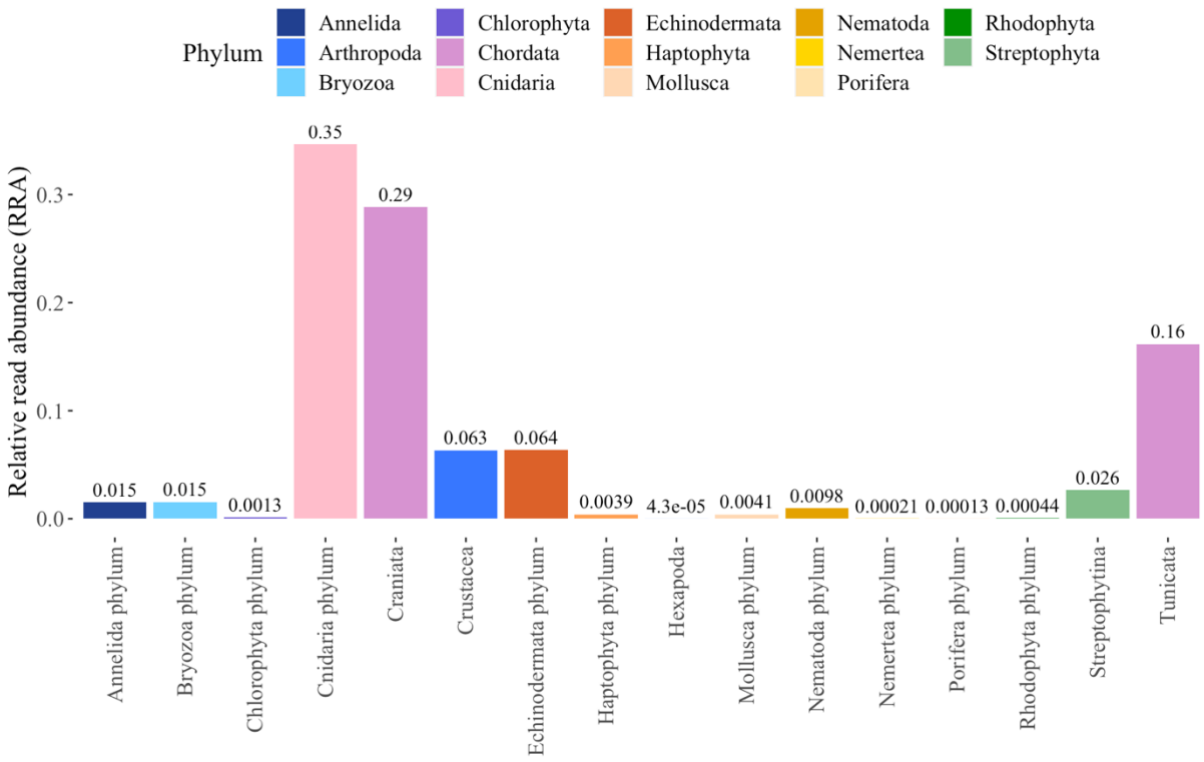

Figure 3.4: Bar plot showing the subphyla and ASV relative read abundance (RRA) averaged by sample and summed by each subphylum. Bars are colored by phylum. The values above the bars indicate the cumulative averaged RRAs for ASVs belonging to each subphylum.

(6.36%), and the Crustacea subphylum (6.33%). A phylogenetic tree of ASVs belonging to Cnidaria and Echinodermata phyla, and Craniata, Tunicata, and Crustacea subphyla is presented in Figure 3.6. No ASV was detected across all samples, but the ASVs occurring at highest frequencies across all samples were assigned to the Anthoatecata order (62%), *Cyanea* genus (54%), Ascidiidae family (42%), Gadiformes order (39%), and Actinopteri class (34%) (see Figure 3.5 and Table 3.4). saRRA values and the frequency of occurrence (FOO) for each ASV with saRRA > 2% are shown in Table 3.4. The relationship between the FOO and saRRA (Figure 3.7) indicates that most ASVs with high relative read abundances averaged across samples were also occurring in many samples.

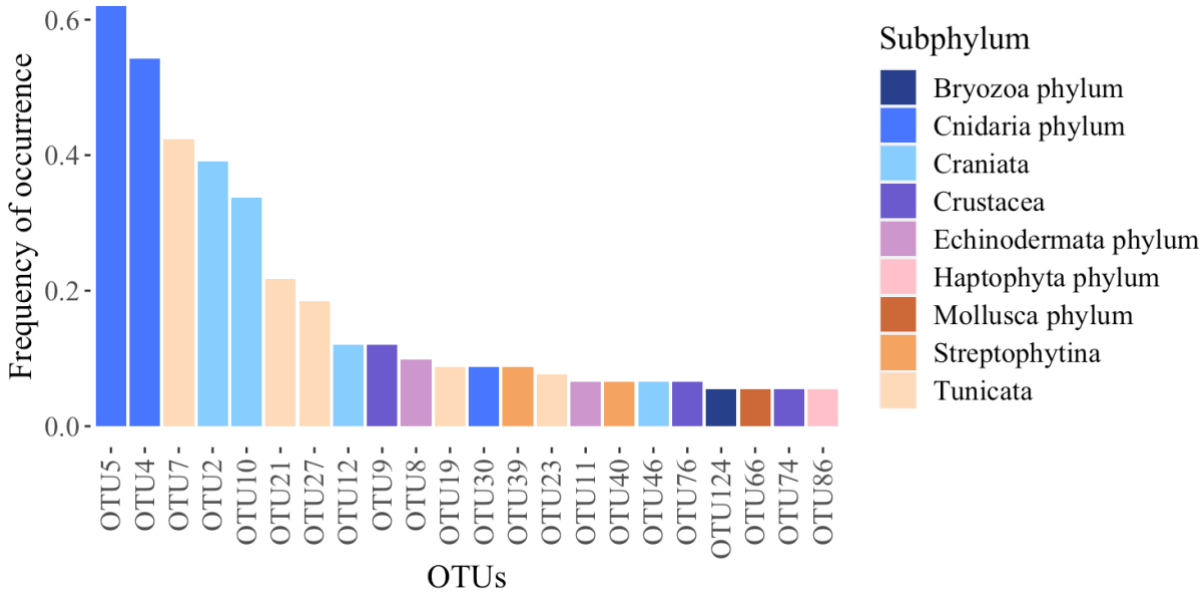

Figure 3.5: ASV (OTU in the plot) frequency of occurrence (FOO) (occurrence > 5%) with a prevalence threshold of 0.01 ranked by subphylum. A few ASVs from the Cnidaria phylum, Tunicata subphylum and Craniata subphylum had the highest FOOs. The bars are ordered from highest to lowest ASV FOO.

The most prevalent ASVs were ASV5 (Anthoathecata order) and ASV4 (*Cyanea* genus) from Cnidaria, ASV2 (Gadiformes order) and ASV10 (Actinopteri class) from the Craniata subphylum, and ASV7 (Ascidiidae family), ASV21 (Ascidiacea class) and ASV27 (*Oikopleura dioica*) from Tunicata (Figure 3.6). ASVs from Echinodermata and Crustacea occurred in fewer samples. The Crustacea subphylum was represented by many ASVs, indicating a lot of lobster forage diversity in this subphylum.

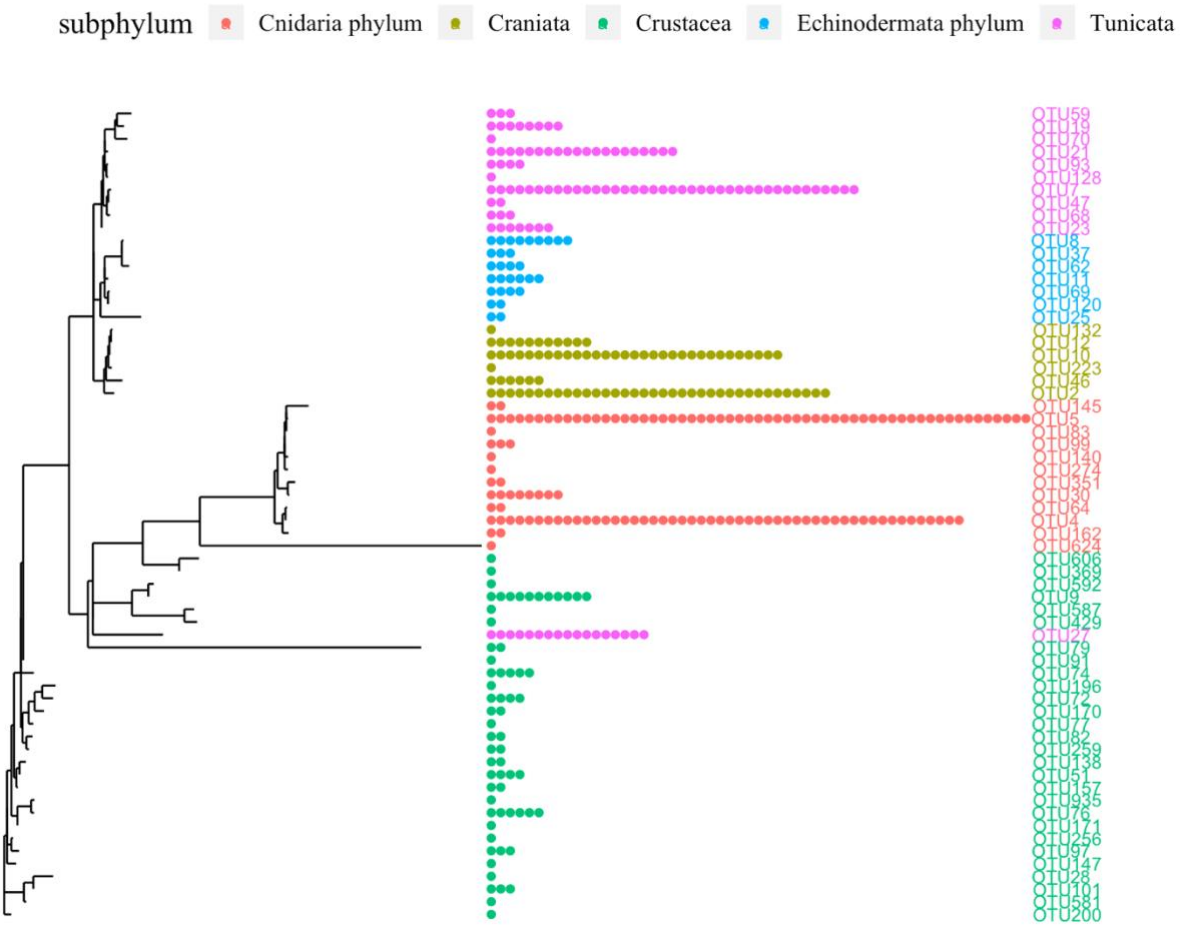

Figure 3.6: Graphical representation of the maximum likelihood phylogenetic tree calculated from the alignment of sequences in the dataset. The plot shows ASV (OTU in the plot) taxonomic identity belonging to the Cnidaria and Echinodermata phyla, and Craniata, Crustacea, and Tunicata subphyla. Each colored dot to the left of an ASV represents a sample that the ASV occurs in. ASV names and dots are colored by subphylum. Branch length indicates amount of genetic change.

Table 3.4: Relative read abundance averaged across samples (saRRA) and occurrence (with frequencies of occurrence (FOO) in brackets) for ASVs with saRRA > 2%.

ASV	Taxonomic designation	Occurrence [FOO]	saRRA
ASV2	Gadiformes order	36 [0.391]	0.172
ASV5	Anthoathecata order	57 [0.620]	0.166
ASV4	*Cyanea* genus	50 [0.543]	0.150
ASV10	Actinopteri class	31 [0.337]	0.0773
ASV7	Ascidiidae family	39 [0.424]	0.0629
ASV9	Decapoda order	11 [0.120]	0.0310
ASV12	Actinopteri class	11 [0.120]	0.0302
ASV19	Molgula genus	8 [0.0870]	0.0225
ASV21	Ascidiacea class	20 [0.217]	0.0219
ASV30	Actiniaria order	8 [0.0870]	0.0202
ASV23	Phlebobranchia order	7 [0.0761]	0.0200

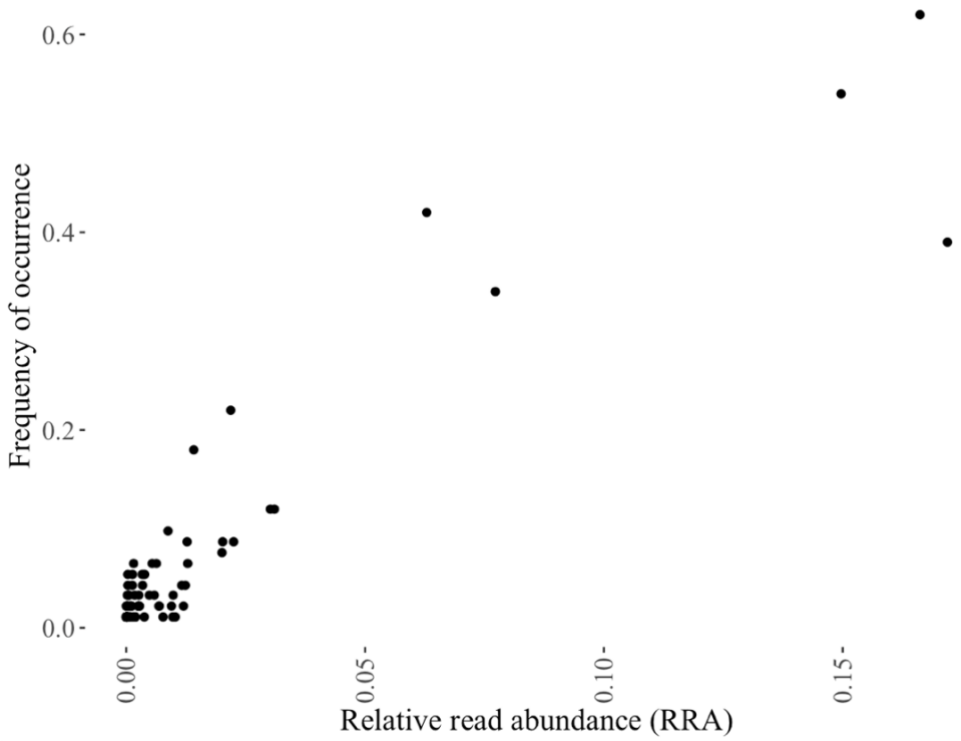

Figure 3.7: ASV relative read abundance (RRA) plotted against frequency of occurrence (FOO) indicates a relationship between the two methods of transforming sequencing reads to dietary information.

3.5.2 Diet variation between BACI groups

The PCoAs of common ASVs (prevalence > 5%) (Figure 3.8) and total ASVs displayed no structure in the dataset that would indicate a difference in Lillesand *H. gammarus* diets between the 2022 reserve and spatial and temporal controls. A slight difference in groups could be observed between samples from 2020 and 2022, which implies a diet difference between years. The principal components explained approximately the same amount of variation in the dataset of common ASVs (61.3%), and the total dataset (60.3%). According to the PERMANOVA, year was a significant factor when testing the common ($F_{(1, 88)} = 5.45$, $p = 0.001$) and total ($F_{(1, 88)} = 4.96$, $p = 0.003$) ASVs. Treatment and the interaction between year and treatment were non-significant for both common (treatment: $F_{(1, 88)} = 1.42$, $p = 0.195$, interaction: $F_{(1, 88)} = 0.677$, $p = 0.622$) and total (treatment: $F_{(1, 88)} = 1.26$, $p = 0.264$, interaction: $F_{(1, 88)} = 0.796$, $p = 0.524$) ASVs. The pairwise test also revealed significant p-values only between groups with differing year levels (before & after), also signifying a diet difference between sampling years. PERMDISP results were non-significant for common ($F_{(3, 88)} = 1.12$, $p = 0.346$) and total ($F_{(3, 88)} = 1.40$, $p = 0.249$) ASVs, indicating significant PERMANOVA results were not simply driven by variation in group dispersion.

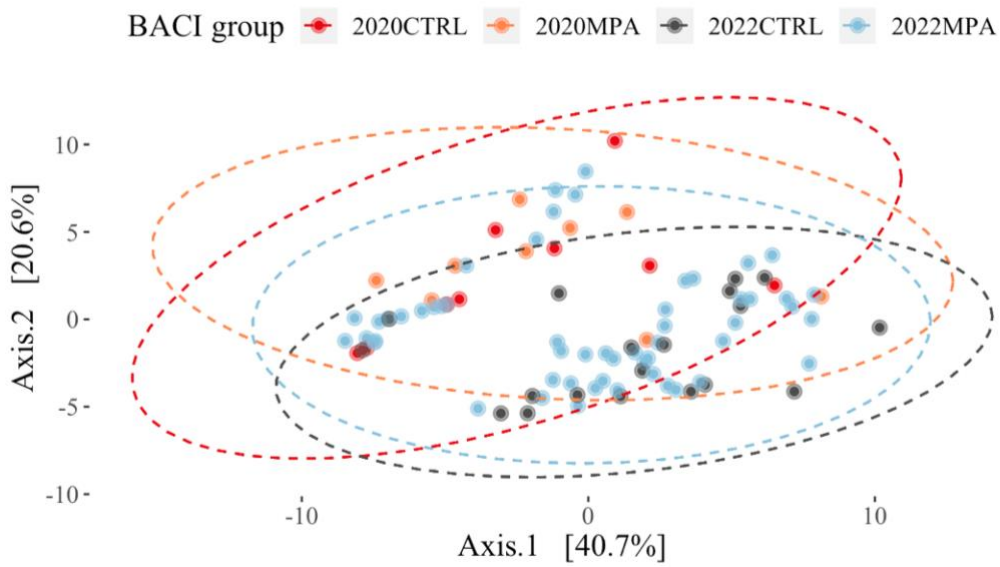

Figure 3.8: PCoA plot of philr transformed data of common ASVs (prevalence > 5%) in the *H. gammarus* diet by BACI groups. Each dot represents a sample belonging to one of four BACI groups: Before Control (red), Before MPA (orange), After Control (black), and after MPA (blue) and are surrounded by 95% confidence ellipses. Principal components each explain 40.7 and 20.6% of the variance.

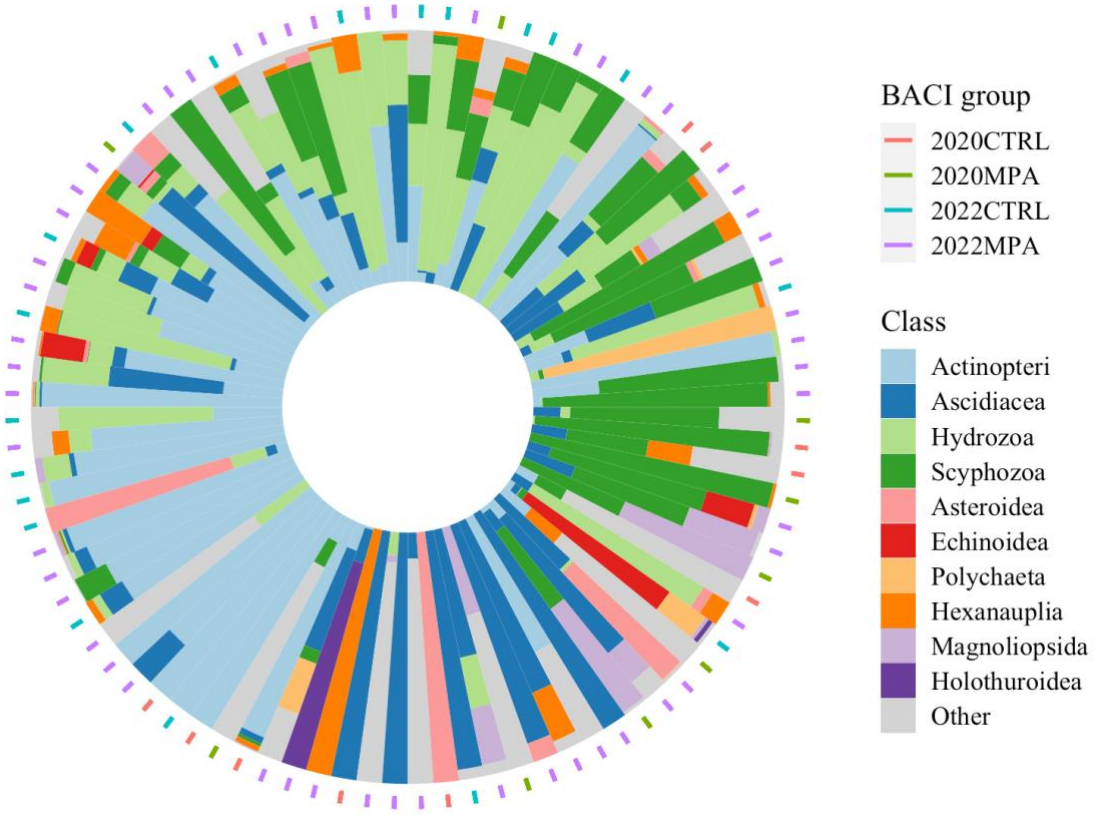

Figure 3.9: Iris plot of centered log transformed (clr) data showing the relative read abundance (RRA) of ASVs assigned to taxa ranked by class for each sample. The BACI group from which each sample was collected is illustrated by colored ticks outside of the ring.

The iris plot (Figure 3.9) indicates some difference in the lobster diet among BACI groups as one half of the ring is highly dominated by samples from 2022. The other half contains very few samples from the 2022 control area. Samples from the 2022 reserve are evenly distributed around the ordinated bar chart. It does not appear that samples from the 2022 reserve had a different diet composition than samples from the temporal and spatial controls.

3.5.3 Demographic variation in dietary composition

According to the PERMANOVA, sex (male (n = 39) and female (n = 52)) and size (1st (n = 15), mid (n = 53) and 3rd (n = 24) quantile of lobster TL) variables were non-significant for datasets of total ASVs, common ASVs, or ASVs belonging to taxa with hard exoskeletons. Furthermore, there was no indication of demographic diet difference according to PCoA plots. Relative read abundance plots had no pattern between different size or sex groups that would suggest that these variables significantly influence the *H. gammarus* diet.

3.5.4 Characteristics of the *H. gammarus* diet

The average number of ASVs found per sample was 5.28 ± 0.406 (S.E.), although as expected there was a correlation between the number of ASVs present in a sample and different sequencing depths and replicate filtering thresholds (Appendix F). The RRA of ASVs per sample ranked by subphylum (Figure 3.10) and the lowest taxon identified of each ASV (Figure 3.11) indicate that the taxonomic composition was diverse and highly variable between

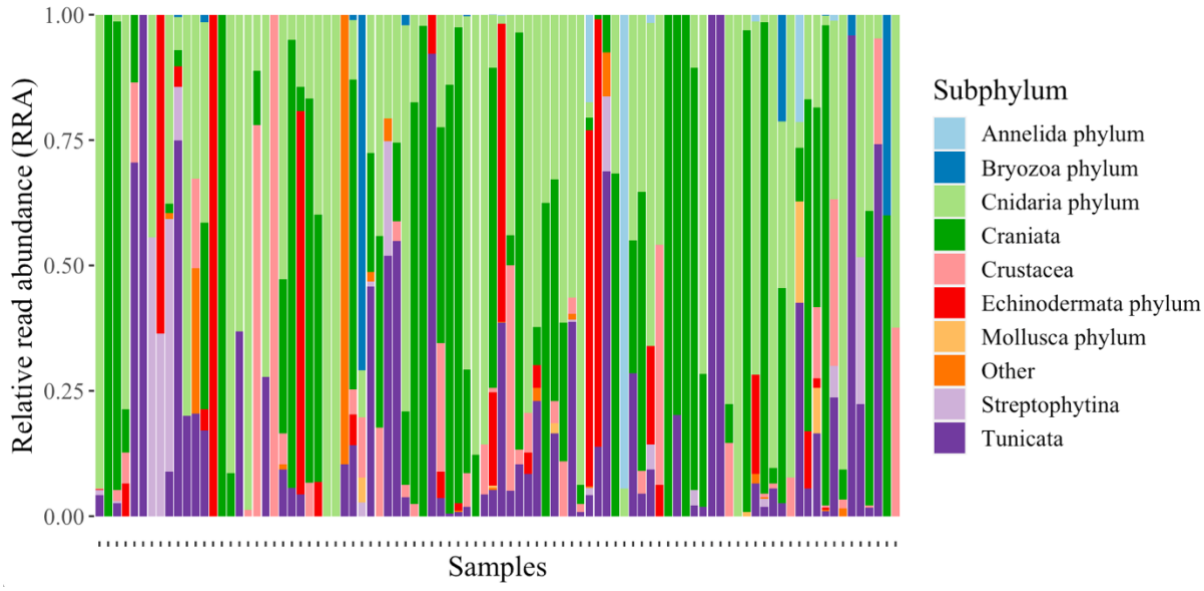

Figure 3.10: Dietary composition of individual fecal samples using relative read abundance (RRA) of each ASV grouped by subphylum. ASVs with a prevalence < 5% and detection level at < 0.5% were aggregated into 'Other' (orange).

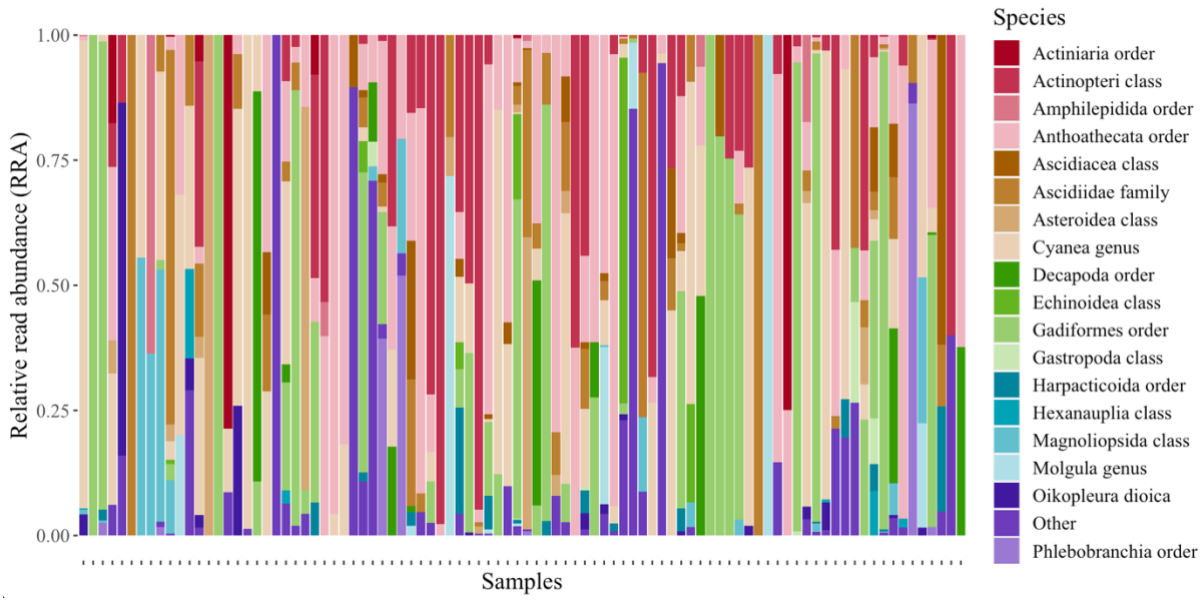

Figure 3.11: Dietary composition of individual fecal samples using relative read abundance (RRA) of each ASV grouped by the lowest taxon identified of each ASV. ASVs with a prevalence < 5% and detection level at < 0.5% were aggregated into 'Other' (purple).

samples. ASVs with a prevalence < 5% and detection level < 0.5% were grouped into 'Other'. The RRA of ASVs assigned to the Gadiformes order was generally high for most samples containing ASVs from the taxon. Many samples had small to moderate RRAs of ASVs from the Tunicata subphylum. Cnidaria was present in most samples at substantial proportions (> 30%). Annelida, Bryozoa, and Mollusca occurred only sporadically in very few samples. ASVs from the Streptophytina phylum had moderate RRAs in a few samples. A few samples were dominated by Echinodermata and Crustacea ASVs present at high RRAs.

The 92 fecal samples contained ASVs belonging to three groups of green algae, four groups of red algae and two groups of phytoplankton with a total algae saRRA of 3.19%. Each ASV assigned to algae taxa was present in only one sample except for the ASVs assigned to the Noelaerhabdaceae family (coccolithophorids) which was found in five samples. Only one ASV assigned to Bivalvia (*Lyonsia norvegica*) was found in one sample. The two ASVs assigned to the Gastropoda class were each present in one and five samples. There were twenty ASVs belonging to the copepod group which were present in between one and five samples, suggesting *H. gammarus* consumes a large diversity of copepod species (Figure 3.12). Of the six ASVs assigned to fish taxa: ASV2, ASV12, and ASV10, had high saRRAs and FOOs (Table 3.4), indicating they came from important prey.

ASVs assigned to taxa with hard exoskeletons (crustaceans, sea urchins, mollusks) had a summarized saRRA of 8.01%. The combined saRRA of organisms regarded as soft-bodied organisms unlikely to be detected by traditional methods after reaching the stomach of *H. gammarus* (cnidarians, tunicates, nematodes, a nemertean worm, and a nudibranch) was 51.8%. Remaining organisms lacking hard exoskeletons but having hard parts or tough tissue expected to be partly conserved after passing to the stomach (polychaetas, sponge, bryozoans, fish, algae, terrestrial plants, and echinoderms excluding sea urchins) had a cumulative saRRA of 40.2%. Lists of taxa within the three groups are provided in Appendix D.

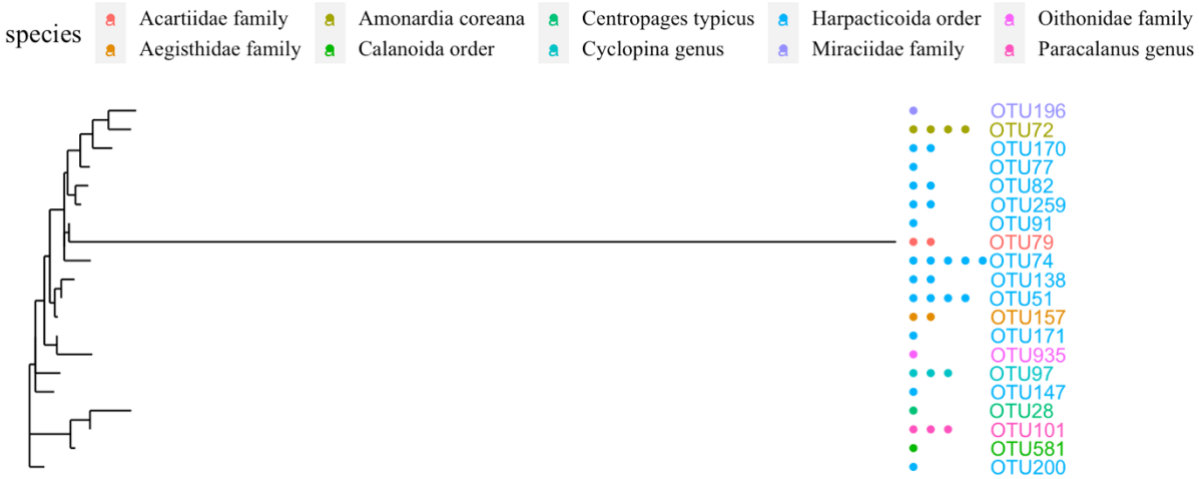

Figure 3.12: Phylogenetic tree (based on the maximum likelihood phylogenetic tree) of copepod ASVs (OTU in the plot) colored by species. Each dot represents a sample the ASV is present in. ASVs are colored by the taxonomic group they belong to. Branch length indicates amount of genetic change.

Blasting ASV2 (Gadiformes order) (BLASTn tool, NCBI) gave a 100% identity match with *Gadus morhua* (Atlantic cod). *G. morhua* was caught as bycatch several times during experimental fishing, and as other species with a high match were from the Gadiformes order, all with a 99.29% match, the sequences were most likely from *G. morhua*. The predicted match for ASV12 using BLASTn (Actinopteri class) was *Labrus bergylta* from the wrasse family (Labridae). Other species also received 100% identity match, yet *Symphodus melops*, also from the wrasse family, was the only one found in temperate East-Atlantic waters. Several individuals and species of wrasse were fished as bycatch during lobster fishing, which indicates that the ASV12 sequence belonged to a member of the Labridae family. ASV23 (Phleobranchia order) was a 100% identity match with the soft tunicate *Ciona intestinalis*. ASV39 (Magnoliopsida class) received a 100% identity score with *Zostera marina* and *Zostera noltei* (eelgrass), along with Eukaryota sp. and a seagrass species from the Pacific Ocean. ASV39

was found in eight samples and made up 1.27% of the saRRAs. ASV35 (Magnoliopsida class) was predicted to be from a terrestrial plant, and ASV40 (Magnoliopsida class) was most likely *Quercus robur* from the oak tree family growing wildly in Southern Norway.

4. Discussion

This study is the first to use DNA-based methods to describe the diet of *H. gammarus* and use the diet information to assess the community response to lobster reserve establishment. One hundred and one ASVs assigned to metazoan taxa in 92 fecal samples were successfully detected, providing a description of *H. gammarus* diet consisting of a wide range of taxa. Many 18S sequences were discarded in filtering steps due to poor sequencing quality (see Appendix E for example). The failed attempt of using the COI marker with blocking primers further limited the number of sequences that could be obtained from the fecal samples. As blocking primers could not be designed for the 18S marker and *H. gammarus* DNA dominated the dataset (91.69%), the detection of rare taxa was likely reduced (Vestheim & Jarman, 2008). The COI marker would also likely provide higher taxonomic resolution due to its more developed reference libraries for metazoan taxa compared to the reference libraries of the 18S marker. Additionally, many samples and ASVs were omitted due to choosing robust sequencing depth and replicate control filtering parameters (see Appendix F). The dataset nonetheless provided quality information used to 1) assess whether diet composition changed within the lobster reserve after two years, 2) determine if diet composition varied depending on sex and size of the lobster, and 3) explore the general diet of *H. gammarus* and the prevalence of soft-bodied organisms. Although there were no significant results indicating an effect of reserve on lobster diet or a difference in diets depending on lobster sex and size class, this study provides novel information of *H. gammarus* diet composition. Using relative read abundance averaged across samples (saRRA) and frequency of occurrence (FOO) as indicators suggests that cnidarians (mainly hydrozoans and 'true jellyfish'), fish, and tunicates were the most important prey items for *H. gammarus*. Other important taxa were echinoderms, crustaceans, and green plants (Streptophytina).

4.1 Diet response to lobster reserve establishment, sex, and size group

The significant difference in *H. gammarus* diet found only between sampling years (common ASVs: $p = 0.001$, total ASVs: $p = 0.003$, PCoA in Figure 3.8) suggests that the lobster reserve had no effect on *H. gammarus* diet within two years of its establishment. The observed difference between 2020 and 2022 was likely the consequence of bottom-up mechanisms caused by natural fluctuations in the abiotic and biotic environment influencing *H. gammarus* food availability (Frederiksen et al., 2006). Indirect effects of marine protection, e.g., changes in trophic interactions and community composition, have been reported to advance

significantly slower than direct effects on the target species (Babcock et al., 2010). Future studies of the reserve might therefore detect changes in *H. gammarus* population structure altering lobster competitive interactions, with flow-on changes in their foraging activity and benthic community composition.

H. gammarus sex had no effect on their diet composition which suggests that males and females have similar foraging strategies. Male lobsters have relatively larger claws than females (Debuse et al., 2001), which is more pronounced within lobster reserves (Sørdalen et al., 2020). Yet no evidence suggests that they consume more organisms that require robust claws to exploit. This indicates that the larger claw size in males is independent of foraging activity and efficiency, supporting studies proposing alternative explanations, e.g., that male claw size is a sexually selected trait (Sørdalen et al., 2018, 2020) important in establishing social dominance (Atema, 1986; Skog, 2009). As the sizes of sampled lobsters ranged from 16.5 to 37.5 cm TL, it is unlikely that the lack of diet differences between *H. gammarus* size classes was the result of a narrow size range. The lack of difference indicates that diet composition is mostly independent of lobster size; that no evidence for intra-specific competition for food was found; and/or that the lobsters are not limited by prey availability at the current population densities. Preliminary results of a study of *H. gammarus* diet observed a diet difference between sizes and found that prey item size correlated with lobster TL (Hallbäck & Warén, 1972). We are unable to support this finding as the DNA-method cannot distinguish between differently sized prey items. The diet of adult *H. gammarus* nevertheless consisted mainly of soft-bodied organisms that should be consumed easily regardless of lobster body and claw size.

4.2 The diet of Lillesand *H. gammarus*

The findings of this study complement previous descriptions of *H. gammarus* and *H. americanus* diet in terms of taxa occurrence. In this study however, prey items were found at different relative abundances as past studies have failed to identify the importance of soft-bodied organisms. Preliminary results from a report of *H. gammarus* diet found that the most important prey groups (based on FOO) were large crustaceans (60.6%), shelled gastropods (36.6%), polychaete worms (23.1%), and unspecified diet items (18.9%), while there were a few occurrences of unspecified fish, calcareous pieces, bivalves, echinoderms, and algae (Hallbäck & Warén, 1972). This is in line with studies of *H. americanus* concluding the lobster's diet consists mainly of gastropods, bivalves, and crustaceans, followed by

echinoderms, and polychaetas (Cox et al., 1997; Elner & Campbell, 1987; Hudon & Lamarche, 1989; Jones & Shulman, 2008; Sainte-Marie & Chabot, 2002). The high saRRA and FOO of soft-bodied organisms such as cnidarians and tunicates in the fecal samples of Lillesand *H. gammarus* indicate that past studies have neglected or largely underestimated their presence in the lobster diet. It is also likely that the importance of fish has been underestimated while hard-shelled organisms, especially large crustaceans, gastropods, and bivalves, have been grossly overestimated. The differences between the discoveries made in this project and current diet descriptions of *H. gammarus* calls for the use and development of the DNA-based diet analysis approach to improve our understanding of the lobster diet composition.

H. gammarus appears to be a generalist predator and scavenger due to the large dietary variation between individuals; consumption of organisms assumed to have been obtained through predation (e.g., tunicates, annelids, polychaetas, echinoderms); and the high relative abundance and prevalence of fish in their diet, likely consumed by scavenging carcasses of adult and juvenile fish. These conclusions complement studies of *H. americanus* and several species of spiny lobster from the Palinuridae family (Carter & Steele, 1982; Cox et al., 1997; Goñi et al., 2001; Joll & Phillips, 1984). Yet, the presence of green and red algae and OTU39 likely from eelgrass in the *H. gammarus* diet indicates that the lobster is omnivorous, which has also been suggested in the literature (Davenport et al., 2021; Jurrius & Rozemeijer, 2022). The low saRRA and occurrence of marine plants might however indicate accidental ingestion during foraging while targeting other prey items (Robeson II et al., 2018). For instance, eelgrass might have been ingested accidentally while foraging in eelgrass meadows harboring many lobster prey organisms such as juvenile fish (Lilley & Unsworth, 2014; Möller et al., 1985), tunicates (Duffy & Harvilicz, 2001), gastropods (Rueda et al., 2009), annelids, nematodes, and nemerteans (Whippo et al., 2018).

A few samples contained low to substantial proportions of plants likely to be terrestrial (flowering plants, conifers). This plant material is assumed to have been transported from land to sea and deposited on the seafloor before being consumed by *H. gammarus* as marine detritus. Marine detritus might have been ingested accidentally by *H. gammarus* while predating on organisms within and among sediments (Redd et al., 2014). However, a study of adult *H. gammarus* found the lobster capable of feeding on and obtaining nutrients from suspended material (Loo et al., 1993), while the southern rock lobster (*Jasus edwardsii*) has been observed eating sediments (Redd et al., 2014), suggesting *H. gammarus* might intentionally ingest

marine detritus. A likely explanation for the prevalence of the surface layer drifting pelagic tunicate *Oikopleura dioica* in the *H. gammarus* diet is consumption of marine detritus containing material from the frequently shed mucus houses of *O. dioica* (Sato et al., 2001). The large diversity of both benthic and pelagic copepods in the *H. gammarus* diet was also probably obtained by feeding on marine detritus containing copepod remains. Although unlikely, as fecal DNA twice digested is expected to be highly degraded, copepods might also have been obtained through secondary predation as their hard exoskeleton would partly protect their tissues and DNA from digestion. If so, a possible explanation for the large diversity of copepods and the high saRRAs of fish ASVs consumed by *H. gammarus* could be ingestion of fish feces containing both fish and prey (copepod) DNA. In support of this hypothesis, the sample composition plot (Figure 3.10) shows that several samples contain both ASVs from the Gadiformes order and Actinopteri class, and the diverse Harpacticoida order of copepods.

H. gammarus individuals might have obtained cnidarian-rich diets by consuming medusae carcasses, which is likely an important food source to benthic scavengers (including crustaceans) (Lebrato et al., 2019; Sweetman et al., 2014). Cnidarians have recently been found to be important prey for many marine predators (Jarman et al., 2013; Lamb et al., 2017; McInnes et al., 2017) although they have been largely overlooked as a prey item due to their low nutritional value (Doyle et al., 2007) and absence in stomach contents of predators because of fast processing of their soft bodies (Arai, 2005). Yet, they contain essential nutrients (Stenvers et al., 2020) particularly in late summer when the nutritious gonads of the medusae develop (Doyle et al., 2007). As samples were only collected in late summer during cnidarian gonad development, it would be interesting to investigate if the proportions of cnidarians in lobster diet vary with seasons depending on their nutrient composition. An alternative or supplementary strategy used by *H. gammarus* to consume cnidarians could be grazing on the polyp stages of the cnidarian life cycle. This has been proposed as the main explanation for the importance of cnidarians to many marine predators' diets (Lamb et al., 2017). As ASVs from coral animals and sea anemones were found in the *H. gammarus* fecal samples, polyp feeding seems like a probable foraging strategy for the lobster. Furthermore, the benthic polyps should prove a more reliable food source to *H. gammarus* than the medusae stage, which would not be expected to produce a constant food supply explaining the high prevalence of cnidarians in the *H. gammarus* diet. The high saRRA of cnidarian ASVs in the fecal samples can be explained by *H. gammarus* needing to consume enough biomass to meet their nutritional requirements (Hays et al., 2018). A study of Chum Salmon found that a high consumption and

fast digestion of cnidarians compensated for their more nutritious and preferred crustacean diet when cnidarians were more available (Arai et al., 2003). Based on sample specific RRAs (Figures 3.10 & 3.11), some lobsters had diets consisting almost exclusively of either fish, tunicates, echinoderms, or crustaceans, while most others had eaten cnidarians in moderate to high relative amounts. This suggests that *H. gammarus* is an opportunist and can replace more nutritious diet items with cnidarians when their preferred prey is scarce or simply when cnidarians are more available.

4.3 Ecosystem implications of *H. gammarus* protection

An outcome of lobster protection described for several lobster species in temporal ecosystems is the increased predation on algae-grazing sea urchins followed by the recovery of kelp forests and macroalgal cover (Babcock et al., 1999, 2010; Ling et al., 2009; Shears & Babcock, 2002). *H. gammarus* might be one of few predators able to penetrate the sea urchins' body armor and could potentially be important for controlling sea urchin populations. The results obtained in this study cannot be used to determine the effect of *H. gammarus* on algae-grazing sea urchins because the sea urchin density within the lobsters' home-range was not measured but believed to be low as their required macroalgae habitat was lacking in the study area. Studying the role of sea urchins as *H. gammarus* prey would provide meaningful information about trophic links and the potential impact of *H. gammarus* on sea urchin populations as a response to lobster reserve establishment. Another ecosystem effect of lobster reserves might be an increase in the ecosystem services provided by *H. gammarus* in its role as a scavenger. The consumption of dead fish and other organisms from the upper water layers contributes to cleaning the seafloor, recycling and conserving energy in the higher trophic levels, and distributing nutrients vertically (Dunlop et al., 2021). Cnidarians might also be affected by recovering populations of *H. gammarus* inside reserves as they were found the most important prey items. Whether *H. gammarus* obtains most of the cnidarians in its diet through scavenging or predation should be explored in future studies as this will reveal the extent of lobster influence on cnidarian populations and the benthic (polyp) stage of their lifecycle.

The results obtained in this study demonstrate the complexity of the *H. gammarus* foraging strategy and trophic role. It seems likely that *H. gammarus* has multiple foraging modes: it is a generalist benthic predator (or omnivore); a scavenger; and a detrivore that consumes organisms opportunistically based on prey availability. As opportunistic feeders their diet

might to some degree reflect the composition and availability of prey in their surrounding community (Baeta et al., 2006), although this results in difficulties determining their trophic role and impact (Cadee, 1984). Seasonal variation in *H. gammarus* activity levels might induce lobsters to become more opportunistic in the winter months when activity is low (Moland, Olsen, Knutsen, et al., 2011). Future studies of *H. gammarus* diet should include data collection during all seasons to gain understanding of the lobster's impact throughout the year. It would also be interesting to compare diet data with eDNA-analysis of water or soil samples, ground-truthing, observational diving or video monitoring programs to investigate if the *H. gammarus* diet composition can be used as an indicator of the community composition. *H. gammarus* can be assumed to occupy a relatively high trophic level in the coastal benthic community as most of its diet items were secondary producers or predators. Additional diet studies of *H. gammarus* carried out in a BACI experimental design, but with reserves monitored over longer timeframes, will contribute to understanding the lobster's ecological role and impact in general, and in response to protection management.

4.4 Methodology and potential

All ways of converting sequencing read data to dietary data introduces some bias (Deagle et al., 2018). This study aimed to get an indication of diet composition for testing differences between groups and explore the general diet of *H. gammarus*, not to determine prey biomass. Thus, results rely mostly on interpretations of RRAs per individual sample and saRRAs summed by groups of ASVs for determining the importance of *H. gammarus* diet items. Weighing each sample equally by averaging RRA across groups likely reduced some of the bias as there were large variations in the number of sequences recovered from each sample (Deagle et al., 2018). Artificial inflation of the importance of rare ASVs was observed using FOO (see Appendix A), yet, supplementing the results with occurrence data of ASVs with high saRRAs provided information of ASV prevalence. Despite the current biases and limitations of DNA metabarcoding (Alberdi et al., 2017; van der Loos & Nijland, 2021) and interpreting sequencing data (Deagle et al., 2018), the methodology used in this project has enabled novel discoveries of *H. gammarus* diet with implications of the lobster's ecology and provided strong indications that the method can be used in determining the effect of lobster reserves over time. This project also emphasizes the huge value of genetic technology in research involving marine ecology and conservation.

5. Conclusions

The DNA-based diet analysis approach used in this study was successful at providing an estimation of the *H. gammarus* diet composition. Based on these dietary data, it can be concluded that two years of protection within a lobster reserve did not affect the Lillesand *H. gammarus* diet. If *H. gammarus* diet is impacted by the benefits associated with lobster reserves, the lack of observable effects was possibly due to the short study period and a slow development of indirect effects stemming from lobster density increase. The approach nonetheless has the potential of identifying diet differences as a response to lobster protection management in future studies if there will be such differences. This study has identified lower taxa not previously found in *H. gammarus* diet, which consisted mainly of hydrozoans, 'true jellyfish', fish, and tunicates, followed by crustaceans and echinoderms. The results of this study suggest that soft-bodied organisms likely to be unidentifiable after passing the *H. gammarus* digestive system are largely underestimated in previous descriptions of *H. gammarus* diet. *H. gammarus* appears to partake in several foraging strategies such as predation, scavenging, omnivory, and detrivory. The species can be described as both a generalist feeder exerting weak trophic effects on a broad range of organisms, and an opportunist feeder likely to reflect the species composition of its surrounding benthic community. Both the successful and unsuccessful approaches attempted in this study demonstrate the potential of molecular methods to uncover knowledge of the ecology of marine organisms and the community responses to protection management.

References

Abesamis, R. A., & Russ, G. R. (2005). Density-Dependent Spillover from a Marine Reserve: Long-Term Evidence. *Ecological Applications*, *15*(5), 1798–1812. https://doi.org/10.1890/05-0174

Acinas, S. G., Sarma-Rupavtarm, R., Klepac-Ceraj, V., & Polz, M. F. (2005). PCR-Induced Sequence Artifacts and Bias: Insights from Comparison of Two 16S rRNA Clone Libraries Constructed from the Same Sample. *Applied and Environmental Microbiology*, *71*(12), 8966–8969. https://doi.org/10.1128/AEM.71.12.8966-8969.2005

Ahlmann-Eltze, C., & Patil, I. (2021). ggsignif: R Package for Displaying Significance Brackets for "ggplot2". *PsyArxiv*. https://doi.org/10.31234/osf.io/7awm6

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2017). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution*, *9*(1), 134–147. https://doi.org/10.1111/2041-210X.12849

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Anderson, M. J., Ellingsen, K. E., & McArdle, B. H. (2006). Multivariate dispersion as a measure of beta diversity. *Ecology Letters*, *9*(6), 683–693. https://doi.org/10.1111/j.1461-0248.2006.00926.x

Arai, M. N. (2005). Predation on pelagic coelenterates: A review. *Journal of the Marine Biological Association of the United Kingdom*, *85*(3), 523–536. https://doi.org/10.1017/S0025315405011458

Arai, M. N., Welch, D. W., Dunsmuir, A. L., Jacobs, M. C., & Ladouceur, A. R. (2003). Digestion of pelagic Ctenophora and Cnidaria by fish. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*(7), 825–829. https://doi.org/10.1139/f03-071

Atema, J. (1986). Review of Sexual Selection and Chemical Communication in the Lobster, *Homarus americanus*. *Canadian Journal of Fisheries and Aquatic Sciences*, *43*(11), 2283–2390. https://doi.org/10.1139/f86-279

Auguie, B. (2017). *_gridExtra: Miscellaneous Functions for "Grid" Graphics_*. (R package version 2.3). https://CRAN.R-project.org/package=gridExtra

Babcock, R. C., Kelly, S., Shears, N. T., Walker, J. W., & Willis, T. J. (1999). Changes in community structure in temperate marine reserves. *Marine Ecology Progress Series*, *189*, 125–134. https://doi.org/10.3354/meps189125

Babcock, R. C., Shears, N. T., Alcala, A. C., Barrett, N. S., Edgar, G. J., Lafferty, K. D., McClanahan, T. R., & Russ, G. R. (2010). Decadal trends in marine reserves reveal differential rates of change in direct and indirect effects. *Proceedings of the National Academy of Sciences*, *107*(43), 18256–18261. https://doi.org/10.1073/pnas.0908012107

Baeta, A., Cabral, H., Marques, J., & Pardal, M. (2006). Feeding ecology of the green crab, *Carcinus maenas* (L., 1758) in a temperate estuary, Portugal. *Crustaceana*, *79*(10), 1181–1193. https://doi.org/10.1163/156854006778859506

Barnett, D. J. m, Arts, I. C. w, & Penders, J. (2021). microViz: An R package for microbiome data visualization and statistics. *Journal of Open Source Software*, *6*(63), 3201. https://doi.org/10.21105/joss.03201

Batuecas, I., Alomar, O., Castañe, C., Piñol, J., Boyer, S., Gallardo-Montoya, L., & Agustí, N. (2022). Development of a multiprimer metabarcoding approach to understanding trophic interactions in agroecosystems. *Insect Science*, *29*(4), 1195–1210. https://doi.org/10.1111/1744-7917.12992

Biro, P. A., & Sampson, P. (2015). Fishing directly selects on growth rate via behaviour: Implications of growth-selection that is independent of size. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1802), 20142283. https://doi.org/10.1098/rspb.2014.2283

Blair, G., Cooper, J., Coppock, A., Humphreys, M., Rudkin, A., & Fultz, N. (2022). *_fabricatr: Imagine Your Data Before You Collect It_*. (R package version 1.0.0). https://CRAN.R-project.org/package=fabricatr

Bonato, L., Peretti, E., Sandionigi, A., & Bortolin, F. (2021). The diet of major predators of forest soils: A first analysis on syntopic species of Chilopoda through DNA metabarcoding. *Soil Biology and Biochemistry*, *158*, 108264. https://doi.org/10.1016/j.soilbio.2021.108264

Boulanger, J., Muresan, L., & Tiemann-Boege, I. (2012). Massively Parallel Haplotyping on Microscopic Beads for the High-Throughput Phase Analysis of Single Molecules. *PLOS ONE*, *7*(4), e36064. https://doi.org/10.1371/journal.pone.0036064

Bradley, R. D., & Hillis, D. M. (1997). Recombinant DNA sequences generated by PCR amplification. *Molecular Biology and Evolution*, *14*(5), 592–593. https://doi.org/10.1093/oxfordjournals.molbev.a025797

Brakenhoff, R. H., Schoenmakers, J. G. G., & Lubsen, N. H. (1991). Chimeric cDNA clones: A novel PCR artifact. *Nucleic Acids Research*, *19*(8), 1949. https://doi.org/10.1093/nar/19.8.1949

Browne, R. M., Mercer, J. P., & Duncan, M. J. (2001). An historical overview of the Republic of Ireland's lobster (*Homarus gammarus* Linnaeus) fishery, with reference to European and North American

(*Homarus americanus* Milne Edwards) lobster landings. *Hydrobiologia*, *465*(1), 49–62. https://doi.org/10.1023/A:1014517614770
Cadee, G. (1984). 'Opportunistic feeding', a serious pitfall in trophic structure analysis of (paleo)faunas. *Lethaia*, *17*, 289–292. https://doi.org/10.1111/j.1502-3931.1984.tb00674.x
Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), Article 7. https://doi.org/10.1038/nmeth.3869
Carøe, C., & Bohmann, K. (2020). Tagsteady: A metabarcoding library preparation protocol to avoid false assignment of sequences to samples. *Molecular Ecology Resources*, *20*(6), 1620–1631. https://doi.org/10.1111/1755-0998.13227
Carter, J. A., & Steele, D. H. (1982). Stomach contents of immature lobsters (*Homarus americanus*) from Placentia Bay, Newfoundland. *Canadian Journal of Zoology*, *60*(3), 337–347. https://doi.org/10.1139/z82-045
Casey, J. M., Meyer, C. P., Morat, F., Brandl, S. J., Planes, S., & Parravicini, V. (2019). Reconstructing hyperdiverse food webs: Gut content metabarcoding as a tool to disentangle trophic interactions on coral reefs. *Methods in Ecology and Evolution*, *10*(8), 1157–1170. https://doi.org/10.1111/2041-210X.13206
CBD (2021). *Open-Ended Working Group on the Post-2020 Global Biodiversity Framework, First Draft of the Post-2020 Framework.* Convention on Biological Diversity.
Chamberlain, S., & Arendsee, Z. (2023). *_taxizedb: Tools for Working with "Taxonomic" Databases_*. (R package version 0.3.1). https://CRAN.R-project.org/package=taxizedb
Chamberlain, S., & Szocs, E. (2013). taxize—Taxonomic search and retrieval in R. *F1000Research*, *2*(191). https://f1000research.com/articles/2-191/v2
Charif, D., & Lobry, J. R. (2007). SeqinR 1.0-2: A contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis. In *Structural approaches to sequence evolution: Molecules, networks, populations* (pp. 207–232).
Cheng, B. S., Altieri, A. H., Torchin, M. E., & Ruiz, G. M. (2019). Can marine reserves restore lost ecosystem functioning? A global synthesis. *Ecology*, *100*(4), e02617. https://doi.org/10.1002/ecy.2617
Childress, M. J., & Jury, S. H. (2006). Behavior. In *Phillips B. (ed.), Lobsters: Biology, Management, Aquaculture and Fisheries* (1st ed., p. 506). Blackwell Publishing Limited. https://doi.org/10.1002/9780470995969
Christensen, N. L., Bartuska, A. M., Brown, J. H., Carpenter, S., D'Antonio, C., Francis, R., Franklin, J. F., MacMahon, J. A., Noss, R. F., Parsons, D. J., Peterson, C. H., Turner, M. G., & Woodmansee, R. G. (1996). The Report of the Ecological Society of America Committee on the Scientific Basis for Ecosystem Management. *Ecological Applications*, *6*(3), 665–691. https://doi.org/10.2307/2269460
Cox, C., Hunt, J. H., Lyons, W. G., & Davis, G. E. (1997). Nocturnal foraging of the Caribbean spiny lobster (*Panulirus argus*) on offshore reefs of Florida, USA. *Marine and Freshwater Research*, *48*(8), 671–680. https://doi.org/10.1071/mf97198
Davenport, J., Jessopp, M., Harman, L., Micaroni, V., & McAllen, R. (2021). Diurnal and nocturnal scavenger communities differ at two shallow-water depths in an Irish marine lough. *Estuarine, Coastal and Shelf Science*, *262*, 107580. https://doi.org/10.1016/j.ecss.2021.107580
Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*(1), 226. https://doi.org/10.1186/s40168-018-0605-2
De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., & Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Molecular Ecology Resources*, *14*(2), 306–323. https://doi.org/10.1111/1755-0998.12188
Deagle, B., Chiaradia, A., McInnes, J., & Jarman, S. (2010). Pyrosequencing faecal DNA to determine diet of little penguins: Is what goes in what comes out? *Conserv. Genet*, *11*, 2039–2048. https://doi.org/10.1007/s10592-010-0096-6
Deagle, B., Eveson, J. P., & Jarman, S. N. (2006). Quantification of damage in DNA recovered from highly degraded samples—A case study on DNA in faeces. *Frontiers in Zoology*, *3*, 11. https://doi.org/10.1186/1742-9994-3-11
Deagle, B., Kirkwood, R., & Jarman, S. (2009). Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology*, *18*, 2022–2038. https://doi.org/10.1111/j.1365-294X.2009.04158.x
Deagle, B., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., Kartzinel, T. R., & Eveson, J. P. (2018). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology*, *28*(2), 391–406. https://doi.org/10.1111/mec.14734

Debuse, V. J., Addison, J. T., & Reynolds, J. D. (2001). Morphometric variability in UK populations of the European lobster. *Journal of the Marine Biological Association of the United Kingdom*, *81*(3), 469–474. https://doi.org/10.1017/S0025315401004106

Díaz, D., Mallol Martínez, S., Parma, A., & Goñi, R. (2011). Decadal trend in lobster reproductive output from a temperate marine protected area. *Marine Ecology Progress Series*, *433*, 149–157. https://doi.org/10.3354/meps09182

Djurhuus, A., Closek, C. J., Kelly, R. P., Pitz, K. J., Michisaki, R. P., Starks, H. A., Walz, K. R., Andruszkiewicz, E. A., Olesin, E., Hubbard, K., Montes, E., Otis, D., Muller-Karger, F. E., Chavez, F. P., Boehm, A. B., & Breitbart, M. (2020). Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nature Communications*, *11*(1), Article 1. https://doi.org/10.1038/s41467-019-14105-1

Dowle, M., & Srinivasan, A. (2023). *_data.table: Extension of `data.frame`_*. (R package version 1.14.8). https://CRAN.R-project.org/package=data.table

Doyle, T. K., Houghton, J. D. R., McDevitt, R., Davenport, J., & Hays, G. C. (2007). The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition. *Journal of Experimental Marine Biology and Ecology*, *343*(2), 239–252. https://doi.org/10.1016/j.jembe.2006.12.010

Duffy, J., & Harvilicz, A. (2001). Species-specific impacts of grazing amphipods in an eelgrass-bed community. *Marine Ecology Progress Series*, *223*, 201–211. https://doi.org/10.3354/meps223201

Dunlop, K., Renaud, P. E., Berge, J., Jones, D. O. B., Harbour, R. P., Tandberg, A. H. S., & Sweetman, A. K. (2021). Benthic scavenger community composition and carrion removal in Arctic and Subarctic fjords. *Polar Biology*, *44*(1), 31–43. https://doi.org/10.1007/s00300-020-02773-5

Dunshea, G. (2009). DNA-Based Diet Analysis for Any Predator. *PLOS ONE*, *4*(4), e5252. https://doi.org/10.1371/journal.pone.0005252

Dunshea, G., Barros, N. B., Berens McCabe, E. J., Gales, N. J., Hindell, M. A., Jarman, S. N., & Wells, R. S. (2013). Stranded dolphin stomach contents represent the free-ranging population's diet. *Biology Letters*, *9*(3), 20121036. https://doi.org/10.1098/rsbl.2012.1036

Elner, R. W., & Campbell, A. (1987). Natural diets of lobster *Homarus americanus* from barren ground and macroalgal habitats off southwestern Nova Scotia, Canada. *Marine Ecology Progress Series*, *37*, 131–140. https://doi.org/10.3354/meps037131

Estes, J. A., & Duggins, D. O. (1995). Sea Otters and Kelp Forests in Alaska: Generality and Variation in a Community Ecological Paradigm. *Ecological Monographs*, *65*(1), 75–100. https://doi.org/10.2307/2937159

Estes, J. A., & Palmisano, J. F. (1974). Sea otters: Their role in structuring nearshore communities. *Science (New York, N.Y.)*, *185*(4156), 1058–1060. https://doi.org/10.1126/science.185.4156.1058

Fernández-Chacón, A., Moland, E., Espeland, S. H., & Olsen, E. M. (2015). Demographic effects of full vs. partial protection from harvesting: Inference from an empirical before–after control-impact study on Atlantic cod. *Journal of Applied Ecology*, *52*(5), 1206–1215.

Foster, Z., Sharpton, T., & Grünwald, N. (2017). Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLOS Computational Biology*, *13*(2), 1–15. https://doi.org/10.1371/journal.pcbi.1005404

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression, Third edition.* https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Frantzen, M. a. J., Silk, J. B., Ferguson, J. W. H., Wayne, R. K., & Kohn, M. H. (1998). Empirical evaluation of preservation methods for faecal DNA. *Molecular Ecology*, *7*(10), 1423–1428. https://doi.org/10.1046/j.1365-294x.1998.00449.x

Franz, N. (2006). *Lobster Markets* (Vol. 87). FAO. https://www.fao.org/fishery/en/publications/76510

Frederiksen, M., Edwards, M., Richardson, A. J., Halliday, N. C., & Wanless, S. (2006). From plankton to top predators: Bottom-up control of a marine food web across four trophic levels. *Journal of Animal Ecology*, *75*(6), 1259–1268. https://doi.org/10.1111/j.1365-2656.2006.01148.x

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications*, *8*(1), Article 1. https://doi.org/10.1038/s41467-017-01312-x

Giakoumi, S., Scianna, C., Plass-Johnson, J., Micheli, F., Grorud-Colvert, K., Thiriet, P., Claudet, J., Di Carlo, G., Di Franco, A., Gaines, S. D., García-Charton, J. A., Lubchenco, J., Reimer, J., Sala, E., & Guidetti, P. (2017). Ecological effects of full and partial protection in the crowded Mediterranean Sea: A regional meta-analysis. *Scientific Reports*, *7*(1), Article 1. https://doi.org/10.1038/s41598-017-08850-w

Glenn, T. C., Nilsen, R. A., Kieran, T. J., Sanders, J. G., Bayona-Vásquez, N. J., Finger, J. W., Pierson, T. W., Bentley, K. E., Hoffberg, S. L., Louha, S., Garcia-De Leon, F. J., del Rio Portilla, M. A., Reed, K. D., Anderson, J. L., Meece, J. K., Aggrey, S. E., Rekaya, R., Alabady, M., Belanger, M., … Faircloth, B. C. (2019). Adapterama I: Universal stubs and primers for 384 unique dual-indexed or 147,456

combinatorially-indexed Illumina libraries (iTru & iNext). *PeerJ*, *7*, e7755. https://doi.org/10.7717/peerj.7755
Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome Datasets Are Compositional: And This Is Not Optional. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.02224
Goñi, R., Quetglas, A., & Reñones, O. (2001). Diet of the spiny lobster *Palinurus elephas* (Decapoda: Palinuridea) from the Columbretes Islands Marine Reserve (north-western Mediterranean). *Journal of the Marine Biological Association of the United Kingdom*, *81*(2), 347–348. https://doi.org/10.1017/S0025315401003861
Gosselin, E., Lonsinger, R., & Waits, L. (2017). Comparing Morphological and Molecular Diet Analyses and Fecal DNA Sampling Protocols for a Terrestrial Carnivore. *Wildlife Society Bulletin*, *41*. https://doi.org/10.1002/wsb.749
Günther, B., Knebelsberger, T., Neumann, H., Laakmann, S., & Martínez Arbizu, P. (2018). Metabarcoding of marine environmental DNA based on mitochondrial and nuclear genes. *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-018-32917-x
Hallbäck, H., & Warén, A. (1972). Food ecology of lobster, *Homarus vulgaris*, in Swedish waters. Some preliminary results. ICES. https://www.ices.dk/sites/pub/CM%20Doccuments/1972/K/1972_K29.pdf
Hardy, C. M., Krull, E. S., Hartley, D. M., & Oliver, R. L. (2010). Carbon source accounting for fish using combined DNA and stable isotope analyses in a regulated lowland river weir pool. *Molecular Ecology*, *19*(1), 197–212. https://doi.org/10.1111/j.1365-294X.2009.04411.x
Hardy, N., Berry, T., Kelaher, B. P., Goldsworthy, S. D., Bunce, M., Coleman, M. A., Gillanders, B. M., Connell, S. D., Blewitt, M., & Figueira, W. (2017). Assessing the trophic ecology of top predators across a recolonisation frontier using DNA metabarcoding of diets. *Marine Ecology Progress Series*, *573*, 237–254. https://doi.org/10.3354/meps12165
Harmelin-Vivien, M., Le Diréach, L., Bayle-Sempere, J., Charbonnel, E., García-Charton, J. A., Ody, D., Pérez-Ruzafa, A., Reñones, O., Sánchez-Jerez, P., & Valle, C. (2008). Gradients of abundance and biomass across reserve boundaries in six Mediterranean marine protected areas: Evidence of fish spillover? *Biological Conservation*, *141*(7), 1829–1839. https://doi.org/10.1016/j.biocon.2008.04.029
Harrell Jr, F. (2023). *_Hmisc: Harrell Miscellaneous_*. (R package version 5.0-1). https://CRAN.R-project.org/package=Hmisc
Hawke, T., Bino, G., Shackleton, M. E., Ross, A. K., & Kingsford, R. T. (2022). Using DNA metabarcoding as a novel approach for analysis of platypus diet. *Scientific Reports*, *12*(1), Article 1. https://doi.org/10.1038/s41598-022-06023-y
Hays, G. C., Doyle, T. K., & Houghton, J. D. R. (2018). A Paradigm Shift in the Trophic Importance of Jellyfish? *Trends in Ecology & Evolution*, *33*(11), 874–884. https://doi.org/10.1016/j.tree.2018.09.001
Hudon, C., & Lamarche, G. (1989). Niche segregation between American lobster *Homarus americanus* and rock crab *Cancer irroratus*. *Marine Ecology Progress Series*, *52*(2), 155–168.
Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2007). MEGAN analysis of metagenomic data. *Genome Research*, *17*(3), 377–386. https://doi.org/10.1101/gr.5969107
Huxley, T. H. (1880). *The crayfish: An introduction to the study of zoology (No. 28). C. Kegan Paul.* (Vol. 28). D. Appleton and Company.
IUCN WCPA (2018). *Applying IUCN's Global Conservation Standards to Marine Protected Areas (MPA). Delivering effective conservation action through MPAs, to secure ocean health & sustainable development. Version 1.0*. Gland, Switzerland. 4pp.
Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., Bradbury, R. H., Cooke, R., Erlandson, J., Estes, J. A., Hughes, T. P., Kidwell, S., Lange, C. B., Lenihan, H. S., Pandolfi, J. M., Peterson, C. H., Steneck, R. S., Tegner, M. J., & Warner, R. R. (2001). Historical Overfishing and the Recent Collapse of Coastal Ecosystems. *Science*, *293*(5530), 629–637. https://doi.org/10.1126/science.1059199
Jarman, S. N., McInnes, J. C., Faux, C., Polanowski, A. M., Marthick, J., Deagle, B. E., Southwell, C., & Emmerson, L. (2013). Adélie Penguin Population Diet Monitoring by Analysis of Food DNA in Scats. *PLOS ONE*, *8*(12), e82227. https://doi.org/10.1371/journal.pone.0082227
Jaureguiberry, P., Titeux, N., Wiemers, M., Bowler, D. E., Coscieme, L., Golden, A. S., Guerra, C. A., Jacob, U., Takahashi, Y., Settele, J., Díaz, S., Molnár, Z., & Purvis, A. (2022). The direct drivers of recent global anthropogenic biodiversity loss. *Science Advances*, *8*(45), eabm9982. https://doi.org/10.1126/sciadv.abm9982
Jo, H., Ventura, M., Vidal, N., Gim, J.-S., Buchaca, T., Barmuta, L. A., Jeppesen, E., & Joo, G.-J. (2016). Discovering hidden biodiversity: The use of complementary monitoring of fish diet based on DNA barcoding in freshwater ecosystems. *Ecology and Evolution*, *6*(1), 219–232. https://doi.org/10.1002/ece3.1825

Joll, L. M., & Phillips, B. F. (1984). Natural diet and growth of juvenile western rock lobsters *Panulirus cygnus* George. *Journal of Experimental Marine Biology and Ecology*, *75*(2), 145–169. https://doi.org/10.1016/0022-0981(84)90178-3

Jones, P. L., & Shulman, M. J. (2008). Subtidal-intertidal trophic links: American lobsters [*Homarus americanus* (Milne-Edwards)] forage in the intertidal zone on nocturnal high tides. *Journal of Experimental Marine Biology and Ecology*, *361*(2), 98–103. https://doi.org/10.1016/j.jembe.2008.05.004

Jurrius, L. H., & Rozemeijer, M. J. C. (2022). The role of the European lobster (*Homarus gammarus*) in the ecosystem: An inventory as part of a feasibility study for passive fisheries on European lobster *Homarus gammarus* in offshore wind farms. *Wageningen Marine Research*. https://doi.org/10.18174/580457

Jusino, M. A., Banik, M. T., Palmer, J. M., Wray, A. K., Xiao, L., Pelton, E., Barber, J. R., Kawahara, A. Y., Gratton, C., Peery, M. Z., & Lindner, D. L. (2019). An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. *Molecular Ecology Resources*, *19*(1), 176–190. https://doi.org/10.1111/1755-0998.12951

Kassambara, A. (2023). *_ggpubr: "ggplot2" Based Publication Ready Plots_*. (R package version 0.6.0). https://rpkgs.datanovia.com/ggpubr/

Kawamata, S., & Taino, S. (2021). Trophic cascade in a marine protected area with artificial reefs: Spiny lobster predation mitigates urchin barrens. *Ecological Applications*, *31*(6), e02364. https://doi.org/10.1002/eap.2364

Kelly, S., Scott, D., MacDiarmid, A. B., & Babcock, R. C. (2000). Spiny lobster, *Jasus edwardsii*, recovery in New Zealand marine reserves. *Biological Conservation*, *92*(3), 359–369. https://doi.org/10.1016/S0006-3207(99)00109-3

Kleiven, A. R., Espeland, S. H., Stiansen, S., Ono, K., Zimmermann, F., & Olsen, E. M. (2022). Technological creep masks continued decline in a lobster (*Homarus gammarus*) fishery over a century. *Scientific Reports*, *12*(1), Article 1. https://doi.org/10.1038/s41598-022-07293-2

Kleiven, A. R., Olsen, E., & Vølstad, J. (2011). Estimating Recreational and Commercial Fishing Effort for European Lobster *Homarus gammarus* by Strip Transect Sampling. *Marine and Coastal Fisheries Dynamics Management and Ecosystem Science*, *3*, 383–393. https://doi.org/10.1080/19425120.2011.638798

Knutsen, J. A., Kleiven, A. R., Olsen, E. M., Knutsen, H., Espeland, S. H., Sordalen, T. K., Thorbjornsen, S. H., Hutchings, J. A., Fernandez-Chacon, A., Huserbraten, M., Villegas-Rios, D., Halvorsen, K. T., Kleiven, P. J. N., Langeland, T. K., & Moland, E. (2022). Lobster reserves as a management tool in coastal waters: Two decades of experience in Norway. *Marine Policy*, *136*, 104908. https://doi.org/10.1016/j.marpol.2021.104908

Kowalczyk, R., Taberlet, P., Coissac, E., Valentini, A., Miquel, C., Kamiński, T., & Wójcik, J. M. (2011). Influence of management practices on large herbivore diet—Case of European bison in Białowieża Primeval Forest (Poland). *Forest Ecology and Management*, *261*(4), 821–828. https://doi.org/10.1016/j.foreco.2010.11.026

Lahti, L., & Shetty, S. (2017). *Microbiome R package*. https://doi.org/10.18129/B9.bioc.microbiome

Lamb, P. D., Hunter, E., Pinnegar, J. K., Creer, S., Davies, R. G., & Taylor, M. I. (2017). Jellyfish on the menu: MtDNA assay reveals scyphozoan predation in the Irish Sea. *Royal Society Open Science*, *4*(11), 171421. https://doi.org/10.1098/rsos.171421

Lebrato, M., Pahlow, M., Frost, J. R., Küter, M., de Jesus Mendes, P., Molinero, J.-C., & Oschlies, A. (2019). Sinking of Gelatinous Zooplankton Biomass Increases Deep Carbon Transfer Efficiency Globally. *Global Biogeochemical Cycles*, *33*(12), 1764–1783. https://doi.org/10.1029/2019GB006265

Leenhardt, P., Low, N., Pascal, N., Micheli, F., & Claudet, J. (2015). Chapter 9—The Role of Marine Protected Areas in Providing Ecosystem Services. In A. Belgrano, G. Woodward, & U. Jacob (Eds.), *Aquatic Functional Biodiversity* (pp. 211–239). Academic Press. https://doi.org/10.1016/B978-0-12-417015-5.00009-8

Lemon, J. (2006). Plotrix: A package in the red light district of R. *R-News*, *6*(4), 8–12.

Lester, S., & Halpern, B. (2008). Biological responses in marine no-take reserves versus partially protected areas. *Marine Ecology Progress Series*, *367*, 49–56. https://doi.org/10.3354/meps07599

Lilley, R. J., & Unsworth, R. K. F. (2014). Atlantic Cod (*Gadus morhua*) benefits from the availability of seagrass (*Zostera marina*) nursery habitat. *Global Ecology and Conservation*, *2*, 367–377. https://doi.org/10.1016/j.gecco.2014.10.002

Ling, S. D., Johnson, C. R., Frusher, S. D., & Ridgway, K. R. (2009). Overfishing reduces resilience of kelp beds to climate-driven catastrophic phase shift. *Proceedings of the National Academy of Sciences*, *106*(52), 22341–22345. https://doi.org/10.1073/pnas.0907529106

Lizaso, J. L. S., Goñi, R., Reñones, O., Charton, J. a. G., Galzin, R., Bayle, J. T., Jerez, P. S., Ruzafa, A. P., & Ramos, A. A. (2000). Density dependence in marine protected populations: A review. *Environmental Conservation*, *27*(2), 144–158. https://doi.org/10.1017/S0376892900000187

Loo, L., Pihl Baden, S., & Ulmestrand, M. (1993). Suspension feeding in adult *Nephrops norvegicus* (L.) and *Homarus gammarus* (L.) (decapoda). *Netherlands Journal of Sea Research*, *31*(3), 291–297. https://doi.org/10.1016/0077-7579(93)90029-R

Marchal, P., Andersen, B., Caillart, B., Eigaard, O., Guyader, O., Hovgaard, H., Iriondo, A., Le Fur, F., Sacchi, J., & Santurtún, M. (2007). Impact of technological creep on fishing effort and fishing mortality, for a selection of European fleets. *ICES Journal of Marine Science*, *64*(1), 192–209. https://doi.org/10.1093/icesjms/fsl014

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), Article 1. https://doi.org/10.14806/ej.17.1.200

Martinez Arbizu, P. (2017). *_pairwiseAdonis: Pairwise Multilevel Comparison using Adonis_*. (R package version 0.4.1.).

McCauley, D. J., Micheli, F., Young, H., Tittensor, D., Brumbaugh, D., Madin, E., Holmes, K., Smith, J., Lotze, H., DeSalles, P., Arnold, S., & Worm, B. (2010). Acute effects of removing large fish from a near-pristine coral reef. *Marine Biology*, *157*, 2739–2750. https://doi.org/10.1007/s00227-010-1533-2

McInnes, J. C., Alderman, R., Lea, M.-A., Raymond, B., Deagle, B. E., Phillips, R. A., Stanworth, A., Thompson, D. R., Catry, P., Weimerskirch, H., Suazo, C. G., Gras, M., & Jarman, S. N. (2017). High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding. *Molecular Ecology*, *26*(18), 4831–4845. https://doi.org/10.1111/mec.14245

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Miller-ter Kuile, A., Apigo, A., Bui, A., DiFiore, B., Forbes, E. S., Lee, M., Orr, D., Preston, D. L., Behm, R., Bogar, T., Childress, J., Dirzo, R., Klope, M., Lafferty, K. D., McLaughlin, J., Morse, M., Motta, C., Park, K., Plummer, K., … Young, H. (2022). Predator–prey interactions of terrestrial invertebrates are determined by predator body size and species identity. *Ecology*, *103*(5), e3634. https://doi.org/10.1002/ecy.3634

Moland, E., Olsen, E., Andvord, K., Knutsen, J., & Stenseth, N. C. (2011). Home range of European lobster (*Homarus gammarus*) in a marine reserve: Implications for future reserve design. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*, 1197–1210. https://doi.org/10.1139/f2011-053

Moland, E., Olsen, E. M., Knutsen, H., Garrigou, P., Espeland, S. H., Kleiven, A. R., André, C., & Knutsen, J. A. (2013). Lobster and cod benefit from small-scale northern marine protected areas: Inference from an empirical before–after control-impact study. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1754), 20122679. https://doi.org/10.1098/rspb.2012.2679

Moland, E., Olsen, E. M., Knutsen, H., Knutsen, J. A., Enersen, S. E., André, C., & Stenseth, N. C. (2011). Activity patterns of wild European lobster *Homarus gammarus* in coastal marine reserves: Implications for future reserve design. *Marine Ecology Progress Series*, *429*, 197–207. https://doi.org/10.3354/meps09102

Möller, P., Pihl, L., & Rosenberg, R. (1985). Benthic faunal energy flow and biological interaction in some shallow marine soft bottom habitats. *Marine Ecology Progress Series*, *27*, 109–121. https://doi.org/10.3354/meps027109

Moszczynska, A., Locke, S. A., McLAUGHLIN, J. D., Marcogliese, D. J., & Crease, T. J. (2009). Development of primers for the mitochondrial cytochrome c oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. *Molecular Ecology Resources*, *9*(1), 75–82. https://doi.org/10.1111/j.1755-0998.2009.02634.x

Mumby, P. J., Harborne, A. R., Williams, J., Kappel, C. V., Brumbaugh, D. R., Micheli, F., Holmes, K. E., Dahlgren, C. P., Paris, C. B., & Blackwell, P. G. (2007). Trophic cascade facilitates coral recruitment in a marine reserve. *Proceedings of the National Academy of Sciences*, *104*(20), 8362–8367. https://doi.org/10.1073/pnas.0702602104

Myers, R. A., Baum, J. K., Shepherd, T. D., Powers, S. P., & Peterson, C. H. (2007). Cascading Effects of the Loss of Apex Predatory Sharks from a Coastal Ocean. *Science*, *315*(5820), 1846–1850. https://doi.org/10.1126/science.1138657

Neuwirth, E. (2022). *_RColorBrewer: ColorBrewer Palettes_*. (R package version 1.1-3). https://CRAN.R-project.org/package=RColorBrewer

Nillos Kleiven, P. J., Espeland, S. H., Olsen, E. M., Abesamis, R. A., Moland, E., & Kleiven, A. R. (2019). Fishing pressure impacts the abundance gradient of European lobsters across the borders of a newly established marine protected area. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1894), 20182455. https://doi.org/10.1098/rspb.2018.2455

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., … Weedon, J. (2022). *vegan: Community Ecology Package* (2.6-4). https://cran.r-project.org/web/packages/vegan/index.html

O'Leary, B. C., Winther-Janson, M., Bainbridge, J. M., Aitken, J., Hawkins, J. P., & Roberts, C. M. (2016). Effective Coverage Targets for Ocean Protection. *Conservation Letters*, *9*(6), 398–404. https://doi.org/10.1111/conl.12247

Pace, M. L., Cole, J. J., Carpenter, S. R., & Kitchell, J. F. (1999). Trophic cascades revealed in diverse ecosystems. *Trends in Ecology & Evolution*, *14*(12), 483–488. https://doi.org/10.1016/S0169-5347(99)01723-1

Paula, D. P., & Andow, D. A. (2023). DNA High-Throughput Sequencing for Arthropod Gut Content Analysis to Evaluate Effectiveness and Safety of Biological Control Agents. *Neotropical Entomology*, *52*(2), 302–332. https://doi.org/10.1007/s13744-022-01011-3

Pauvert, C. (2023). *_psadd: Additions to phyloseq package for microbiome analysis_*. (R package version 0.1.3).

Pinnegar, J. K., Polunin, N. V. C., Francour, P., Badalamenti, F., Chemello, R., Harmelin-Vivien, M.-L., Hereu, B., Milazzo, M., Zabala, M., D'anna, G., & Pipitone, C. (2000). Trophic cascades in benthic marine ecosystems: Lessons for fisheries and protected-area management. *Environmental Conservation*, *27*(2), 179–200. https://doi.org/10.1017/S0376892900000205

Plate, T., & Heiberger, R. (2016). *_abind: Combine Multidimensional Arrays_*. (R package version 1.4-5). https://CRAN.R-project.org/package=abind

Pompanon, F., Coissac, É., & Taberlet, P. (2011). Metabarcoding, a new way of analysing biodiversity. In *Biofutur* (Vol. 319, p. 32).

Pompanon, F., Deagle, B., Symondson, W. O. C., Brown, D. S., Jarman, S. N., & Taberlet, P. (2012). Who is eating what: Diet assessment using next generation sequencing. *Molecular Ecology*, *21*(8), 1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x

R Core Team. (2022). *R: A language and environment for statistical computing*. R. https://www.R-project.org/

Redd, K. S., Ling, S. D., Frusher, S. D., Jarman, S., & Johnson, C. R. (2014). Using molecular prey detection to quantify rock lobster predation on barrens-forming sea urchins. *Molecular Ecology*, *23*(15), 3849–3869. https://doi.org/10.1111/mec.12795

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., & Coissac, E. (2011). ecoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research*, *39*(21), e145. https://doi.org/10.1093/nar/gkr732

Richardson, A. J., Lamberts, C., Isaacs, G., Moloney, C. L., & Gibbons, M. J. (2000). Length-weight relationships for some important forage crustaceans from South Africa. *Naga*, *23*(2), 29–33.

Rinker, T. W. (2021). *textshape: Tools for Reshaping Text* (version 1.7.3). https://github.com/trinker/textshape

Robeson II, M. S., Khanipov, K., Golovko, G., Wisely, S. M., White, M. D., Bodenchuck, M., Smyser, T. J., Fofanov, Y., Fierer, N., & Piaggio, A. J. (2018). Assessing the utility of metabarcoding for diet analyses of the omnivorous wild pig (*Sus scrofa*). *Ecology and Evolution*, *8*(1), 185–196. https://doi.org/10.1002/ece3.3638

Rowley, R. J. (1994). Marine reserves in fisheries management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *4*(3), 233–254. https://doi.org/10.1002/aqc.3270040305

Rozemeijer, M. J. C., & Wolfshaar, K. E. van de. (2019). Desktop study on autecology and productivity of European lobster (*Homarus gammarus*, L) in offshore wind farms. *Wageningen Marine Research*. https://doi.org/10.18174/466861

Rueda, J. L., Gofas, S., Urra, J., & Salas, C. (2009). A highly diverse molluscan assemblage associated with eelgrass beds (*Zostera marina* L.) in the Alboran Sea: Micro-habitat preference, feeding guilds and biogeographical distribution. *Scientia Marina*, *73*(4), Article 4. https://doi.org/10.3989/scimar.2009.73n4679

Sainte-Marie, B., & Chabot, D. (2002). Ontogenetic shifts in natural diet during benthic stages of American lobster (*Homarus americanus*), off the Magdalen Islands. *Fishery Bulletin*, *100*(1), 106–116.

Sakaguchi, S. O., Shimamura, S., Shimizu, Y., Ogawa, G., Yamada, Y., Shimizu, K., Kasai, H., Kitazato, H., Fujiwara, Y., Fujikura, K., & Takishita, K. (2017). Comparison of morphological and DNA-based techniques for stomach content analyses in juvenile chum salmon *Oncorhynchus keta*: A case study on diet richness of juvenile fishes. *Fisheries Science*, *83*(1), 47–56. https://doi.org/10.1007/s12562-016-1040-6

Sala, E., & Giakoumi, S. (2018). No-take marine reserves are the most effective protected areas in the ocean. *ICES Journal of Marine Science*, *75*(3), 1166–1168. https://doi.org/10.1093/icesjms/fsx059

Sato, R., Tanaka, Y., & Ishimaru, T. (2001). House Production by Oikopleura dioica (Tunicata, Appendicularia) Under Laboratory Conditions. *Journal of Plankton Research*, *23*(4), 415–423. https://doi.org/10.1093/plankt/23.4.415

Scheffer, M., Rinaldi, S., & Kuznetsov, Y. A. (2000). Effects of fish on plankton dynamics: A theoretical analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(6), 1208–1219. https://doi.org/10.1139/f00-018

Schliep, K. P. (2011). phangorn: Phylogenetic analysis in R. *Bioinformatics*, *27*(4), 592–593.

Sciberras, M., Jenkins, S. R., Kaiser, M. J., Hawkins, S. J., & Pullin, A. S. (2013). Evaluating the biological effectiveness of fully and partially protected marine areas. *Environmental Evidence*, *2*(1), 4. https://doi.org/10.1186/2047-2382-2-4

Shears, N. T., & Babcock, R. C. (2002). Marine reserves demonstrate top-down control of community structure on temperate reefs. *Oecologia*, *132*(1), 131–142. https://doi.org/10.1007/s00442-002-0920-x

Shears, N. T., Grace, R. V., Usmar, N. R., Kerr, V., & Babcock, R. C. (2006). Long-term trends in lobster populations in a partially protected vs. No-take Marine Park. *Biological Conservation*, *2*(132), 222–231. https://doi.org/10.1016/j.biocon.2006.04.001

Shehzad, W., Riaz, T., Nawaz, M. A., Miquel, C., Poillot, C., Shah, S. A., Pompanon, F., Coissac, E., & Taberlet, P. (2012). Carnivore diet analysis based on next-generation sequencing: Application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. *Molecular Ecology*, *21*(8), 1951–1965. https://doi.org/10.1111/j.1365-294X.2011.05424.x

Sherrill-Mix, S. (2023). *_taxonomizr: Functions to Work with NCBI Accessions and Taxonomy_*. (R package version 0.10.2). https://CRAN.R-project.org/package=taxonomizr

Silverman, J. D., Washburne, A. D., Mukherjee, S., & David, L. A. (2017). A phylogenetic transform enhances analysis of compositional microbiota data. *ELife*, *6*, e21887. https://doi.org/10.7554/eLife.21887

Skog, M. (2009). Intersexual differences in European lobster (*Homarus gammarus*): Recognition mechanisms and agonistic behaviours. *Behaviour*, *146*(8), 1071–1091. https://doi.org/10.1163/156853909X406437

Smith, S. D. (2019). phylosmith: An R-package for reproducible and efficient microbiome analysis with phyloseq-objects. *Journal of Open Source Software*, *4*(38), 1442. https://doi.org/10.21105/joss.01442

Smokorowski, K. E., & Randall, R. G. (2017). Cautions on using the Before-After-Control-Impact design in environmental effects monitoring programs. *FACETS*, *2*, 212–232. https://doi.org/10.1139/facets-2016-0058

Sørdalen, T. K., Halvorsen, K. T., Harrison, H. B., Ellis, C. D., Vøllestad, L. A., Knutsen, H., Moland, E., & Olsen, E. M. (2018). Harvesting changes mating behaviour in European lobster. *Evolutionary Applications*, *11*(6), 963–977. https://doi.org/10.1111/eva.12611

Sørdalen, T. K., Halvorsen, K. T., & Olsen, E. M. (2022). Protection from fishing improves body growth of an exploited species. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1987), 20221718. https://doi.org/10.1098/rspb.2022.1718

Sørdalen, T. K., Halvorsen, K. T., Vøllestad, L. A., Moland, E., & Olsen, E. M. (2020). Marine protected areas rescue a sexually selected trait in European lobster. *Evolutionary Applications*, *13*(9), 2222–2233. https://doi.org/10.1111/eva.12992

Stenvers, V., Chi, X., & Javidpour, J. (2020). Seasonal variability of the fatty acid composition in *Aurelia aurita* (Cnidaria: Scyphozoa): implications for gelativore food web studies. *Journal of Plankton Research*, *42*(4), 440–452. https://doi.org/10.1093/plankt/fbaa026

Sweetman, A. K., Smith, C. R., Dale, T., & Jones, D. O. B. (2014). Rapid scavenging of jellyfish carcasses reveals the importance of gelatinous material to deep-sea food webs. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1796), 20142210. https://doi.org/10.1098/rspb.2014.2210

Symondson, W. O. C. (2002). Molecular identification of prey in predator diets. *Molecular Ecology*, *11*(4), 627–641. https://doi.org/10.1046/j.1365-294X.2002.01471.x

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford University Press. https://doi.org/10.1093/oso/9780198767220.001.0001

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*(8), 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Tandberg, A. H. S., Djursvoll, P., Falkenhaug, T., Glenner, H., Meland, K., & Walseng, B. (2021). Krepsdyr: Vurdering av hummer *Homarus gammarus* for Norge. Rødlista for arter 2021. Artsdatabanken. https://artsdatabanken.no/lister/rodlisteforarter/2021/14133

Troedsson, C., Simonelli, P., Nägele, V., Nejstgaard, J. C., & Frischer, M. E. (2009). Quantification of copepod gut content by differential length amplification quantitative PCR (dla-qPCR). *Marine Biology*, *156*(3), 253–259. https://doi.org/10.1007/s00227-008-1079-8

Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. *Trends in Ecology & Evolution*, *24*(2), 110–117. https://doi.org/10.1016/j.tree.2008.09.011

van der Loos, L. M., & Nijland, R. (2021). Biases in bulk: DNA metabarcoding of marine communities and the methodology involved. *Molecular Ecology*, *30*(13), 3270–3288. https://doi.org/10.1111/mec.15592

Vestheim, H., & Jarman, S. N. (2008). Blocking primers to enhance PCR amplification of rare sequences in mixed samples – a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology*, *5*(1), 12. https://doi.org/10.1186/1742-9994-5-12

Whippo, R., Knight, N. S., Prentice, C., Cristiani, J., Siegle, M. R., & O'Connor, M. I. (2018). Epifaunal diversity patterns within and among seagrass meadows suggest landscape-scale biodiversity processes. *Ecosphere*, *9*(11), e02490. https://doi.org/10.1002/ecs2.2490

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4

Wickham, H. (2022). *_stringr: Simple, Consistent Wrappers for Common String Operations_*. (R package version 1.5.0). https://CRAN.R-project.org/package=stringr

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., … Yutani, H. (2019). "Welcome to the tidyverse." *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wickham, H., François, R., Henry, L., Müller, K., & Vaughan, D. (2023). *_dplyr: A Grammar of Data Manipulation_*. (R package version 1.1.2). https://CRAN.R-project.org/package=dplyr

Wickham, H., Hester, J., Chang, W., & Bryan, J. (2022). *_devtools: Tools to Make Developing R Packages Easier_*. (R package version 2.4.5). https://CRAN.R-project.org/package=devtools

Williams, M. J. (1981). Methods for analysis of natural diet in portunid crabs (Crustacea:Decapoda:Portunidae). *Journal of Experimental Marine Biology and Ecology*, *52*(1), 103–113. https://doi.org/10.1016/0022-0981(81)90174-X

Williamson, D., G.R, R., & Ayling, A. (2004). No-take marine reserves increase abundance and biomass of reef fish on inshore fringing reefs of the Great Barrier Reef. *Environmental Conservation*, *31*, 149–159. https://doi.org/10.1017/S0376892904001262

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., Jackson, J. B. C., Lotze, H. K., Micheli, F., Palumbi, S. R., Sala, E., Selkoe, K. A., Stachowicz, J. J., & Watson, R. (2006). Impacts of Biodiversity Loss on Ocean Ecosystem Services. *Science*, *314*(5800), 787–790. https://doi.org/10.1126/science.1132294

Wright, E. S. (2016). Using DECIPHER v2.0 to Analyze Big Biological Sequence Data in R. *The R Journal*, *8*(1), 352–359.

Wright, K. (2021). *_pals: Color Palettes, Colormaps, and Tools to Evaluate Them_*. (R package version 1.7). https://CRAN.R-project.org/package=pals

Yu, D. W., Ji, Y., Emerson, B. C., Wang, X., Ye, C., Yang, C., & Ding, Z. (2012). Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods in Ecology and Evolution*, *3*(4), 613–623. https://doi.org/10.1111/j.2041-210X.2012.00198.x

Zamora-Terol, S., Novotny, A., & Winder, M. (2020). Reconstructing marine plankton food web interactions using DNA metabarcoding. *Molecular Ecology*, *29*(17), 3380–3395. https://doi.org/10.1111/mec.15555

Zhang, J. (2021). *_phylotools: Phylogenetic Tools for Eco-Phylogenetics_*. (R package version 0.2.4). https://github.com/helixcn/phylotools

Appendix A: Taxonomic table

Table A.1: Taxonomic table of the lowest taxa detected in the *H. gammarus* fecal samples grouped by phylum. Percent relative read abundance averaged by sample (saRRA) (and for some taxa, the percent summed saRRA of ASVs assigned to the same taxon), occurrence and percent frequency of occurrence (FOO) per taxon is presented. Taxa within phyla are sorted from highest to lowest %saRRA.

Phylum	Lowest taxa	saRRA [%]	Occurrence	FOO [%]
Annelida	Polychaeta class	1.03	1	1.09
	Pectinariidae family	0.263	3	3.26
	Spionida order	0.19	1	1.09
	Notomastus genus	0.0181	2	2.17
	Syllidia sp. A ZW-2021	0.000221	1	1.09
Arthropoda	Decapoda order	3.1	11	12.0
	Amonardia coreana	1.16	4	43.5
	Harpacticoida order	0.983	22	23.9
	Hexanauplia class	0.347	7	7.61
	Acartiidae family	0.259	2	2.17
	Cyclopina genus	0.177	1	1.09
	Aegisthidae family	0.113	2	2.17
	Paracalanus genus	0.041	3	3.26
	Centropages typicus	0.0397	1	1.09
	Miraciidae family	0.0356	1	1.09
	Hippolytidae family	0.0278	2	2.17
	Podocopida order	0.0196	2	2.17
	Calanoida order	0.014	1	1.09
	Nephropidae family	0.0095	1	1.09
	Insecta class	0.00434	1	1.09
	Oithonidae family	0.000307	1	1.09
Bryozoa	Cyclostomatida order	0.773	1	1.09
	Cheilostomatida order	0.729	6	6.52
	Triticella flava	0.483	3	3.26
	Gymnolaemata class	0.252	2	2.17
Chlorophyta	Trebouxiophyceae class	0.0958	1	1.09
	Ulotrichales order	0.0166	1	1.09
	Rhizoclonium genus	0.0127	1	1.09
	Tetraselmis genus	0.001	1	1.09
Chordata	Gadiformes order	17.2	36	39.1
	Actinopteri class	11.5	48	53.3
	Molgula genus	2.84	11	12.0
	Ascidiacea class	2.19	20	21.7
	Phlebobranchia order	2	7	7.61
	Oikopleura dioica	1.41	17	18.5

	Ascidiidae family	1.22	44	47.8
	Pleuronectiformes order	0.0939	1	1.09
	Stolidobranchia order	0.0715	5	5.43
	Molgulidae family	0.0096	1	1.09
Cnidaria	Anthoathecata order	17.2	63	68.5
	Cyanea genus	15.1	52	56.5
	Actiniaria order	2.02	8	8.70
	Semaeostomeae order	0.268	2	2.17
	Anthozoa class	0.0524	2	2.17
	Myxozoa class	0.0214	1	1.09
	Leptothecata order	0.0165	2	2.17
Echinodermata	Asteroidea class	3.09	16	17.4
	Echinoidea class	1.29	6	6.52
	Amphilepidida order	1.04	6	6.52
	Apodida order	0.947	2	2.17
Haptophyta	Noelaerhabdaceae family	0.386	5	5.43
Mollusca	Gastropoda class	0.391	6	6.52
	Lyonsia norwegica	0.0018	1	1.09
	Onchidoris muricata	0.0134	1	1.09
Nematoda	Enoplida order	0.974	1	1.09
	Oncholaimidae family	0.0019	2	2.17
	Anticomidae family	0.0016	1	1.09
	Chromadoridae family	0.0014	1	1.09
Nemertea	Heteronemertea order	0.0206	1	1.09
Porifera	Demospongiae class	0.0129	2	2.17
Rhodophyta	Erythrotrichiaceae family	0.0291	1	1.09
	Ceramiaceae family	0.0105	1	1.09
	Rhodomelaceae family	0.0037	1	1.09
	Bonnemaisonia genus	0.0006	1	1.09
Streptophyta	Magnoliopsida class	2.62	20	21.7
	Malpighiales order	0.0165	1	1.09
	Pinopsida class	0.0113	1	1.09

Appendix B: Testing of PCR primers and blocking primers (COI)

PCR primers and blocking primers for the COI marker were tested during experimental qPCR and gel electrophoresis to ensure that lobster DNA was blocked while the DNA of related or relevant species was amplified in the PCR steps.

Four COI specific blocking primers were designed and tested on DNA extracted from *H. americanus* (C), *N. norvegicus* (N), *M. edulis* (B) and *P. borealis* (S). The PCR was set up according to specifications in table G.1. The reaction mixture per reaction consisted of 7.5 µl AmpliGold, 0.25 µl forward PCR primer, 0.25 µl reverse PCR primer, 0.75 µl blocking primer, 0.15 µl BSA, 0.5 µl Sybr green. Molecular grade water was added to obtain a reaction volume of 15 µl per reaction. Thermocycling conditions were 95°C for 10 min, then 35 cycles at 94°C for 1 min, 43°C for 1 min and 72°C for 1 min, followed by 72°C for 5 min. The results of gel electrophoresis of the PCR product are shown in Figure B.1. Results show that PCR primers are successful at amplifying DNA from all samples when no blocking primer is present, while it also shows that blocking primer 1 (Table B.1) was successful at blocking predator DNA amplification without significantly reducing amplification of DNA from related species.

Table B.1: qPCR setup for testing blocking primers on *H. americanus* (C), *N. norvegicus* (N), *M. edulis* (B) and *P. borealis* (S). Negative template control (NTC) tubes lack DNA templates.

	No block	**Block 1**	**Block 2**	**Block 3**	**Block 4**	**NTC**
Tube/well	1	2	3	4	5	6
A	C1	C1	C1	C1	C1	No block
B	C2	C2	C2	C2	C2	Block 1
C	C3	C3	C3	C3	C3	Block 2
D	N1	N1	N1	N1	N1	Block 3
E	N2	N2	N2	N2	N2	Block 4
F	B1	B1	B1	B1	B1	-
G	S1	S1	S1	S1	S1	-
H	S2	S2	S2	S2	S2	-

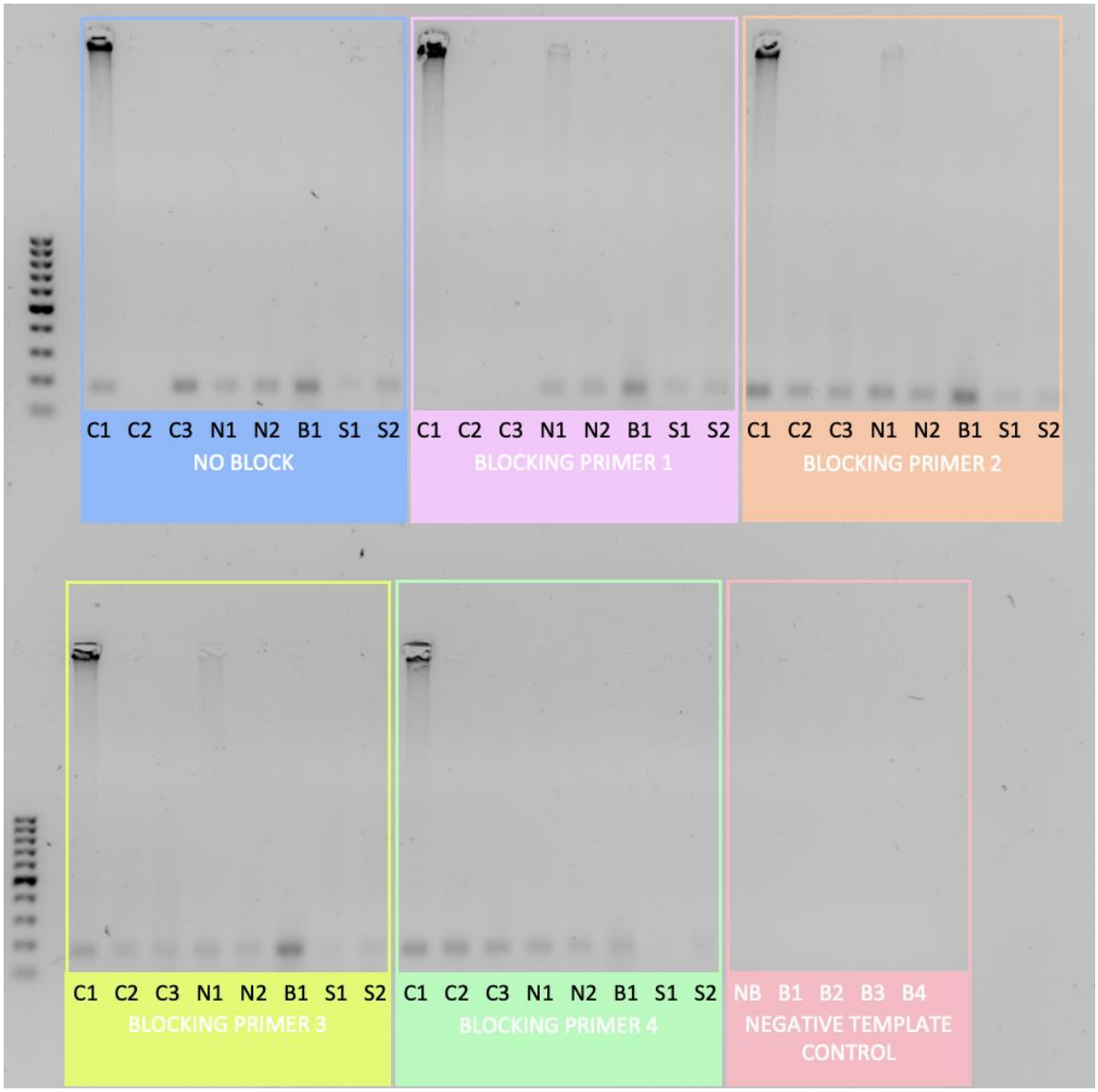

Figure B.1: Gel electrophoresis of PCR products testing four different blocking primers on DNA templates from *H. americanus* (C), *N. norvegicus* (N), *M. edulis* (B) and *P. borealis* (S).

Further testing was done using varying concentrations of blocking primer and DNA to optimize the methods (Table B.2, Figure B.2). A 3x concentration of blocking primer to PCR primer was chosen as it most successfully blocked amplification of *H. americanus* and amplified the *N. norvegicus* DNA.

Table B.2: qPCR setup for testing concentrations of blocking primer and samples C3 and N1.

Row	Sample concentrations	Blocking primer 1 concentrations				
1	C3 1:2	No block	3x	5x	7x	-
2	C3 1:5					No block
3	C3 1:10					NTC 3x
4	C3 1:100					NTC 5x
5	N1 1:2					NTC 7x
6	N1 1:5					-
7	N1 1:10					-
8	N1 1:100					-

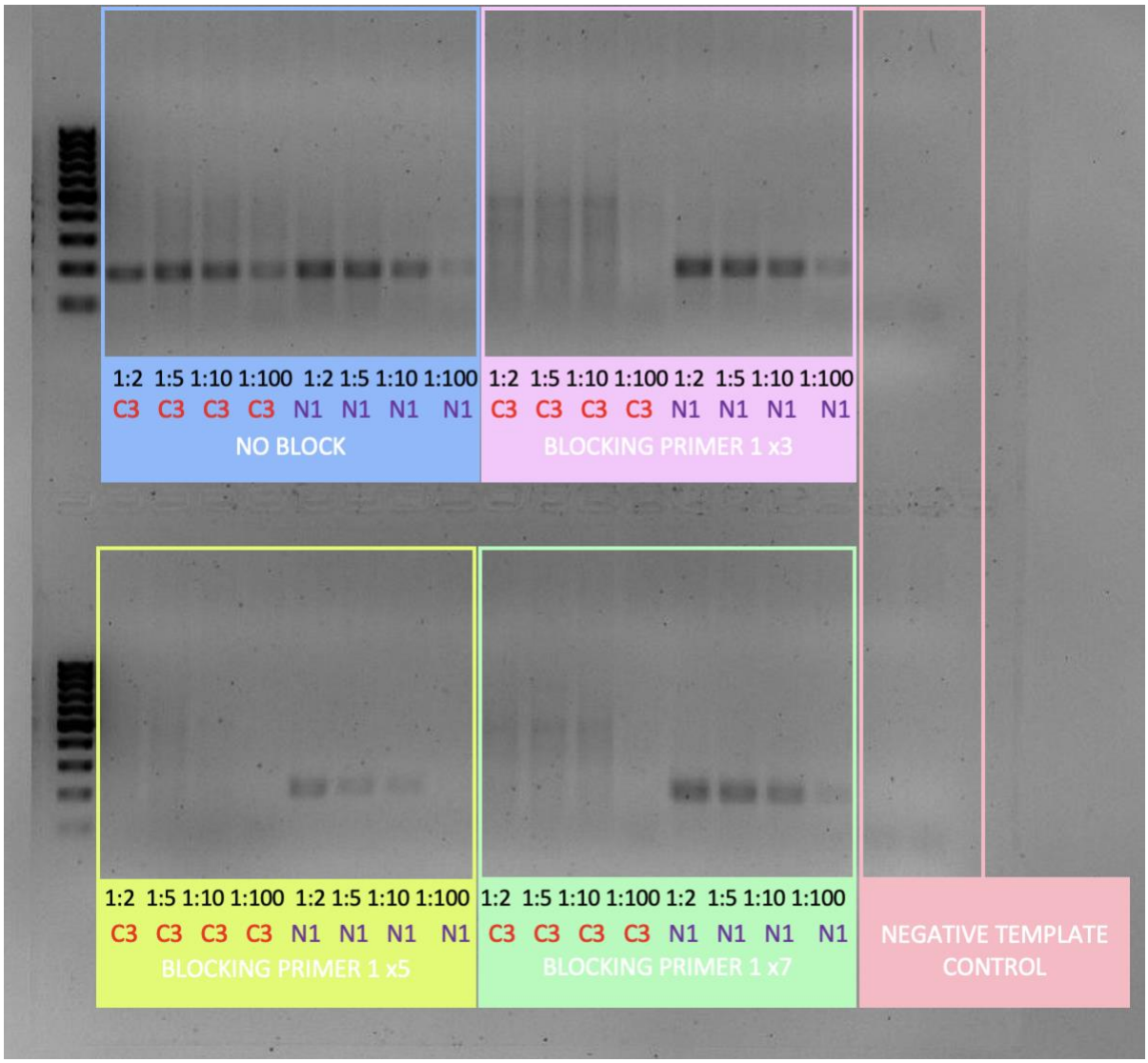

Figure B.2: Agarose gel results for concentration gradient experiment using N. norvegicus (N1) and H. americanus (C3) DNA templates and blocking primer at varying concentrations.

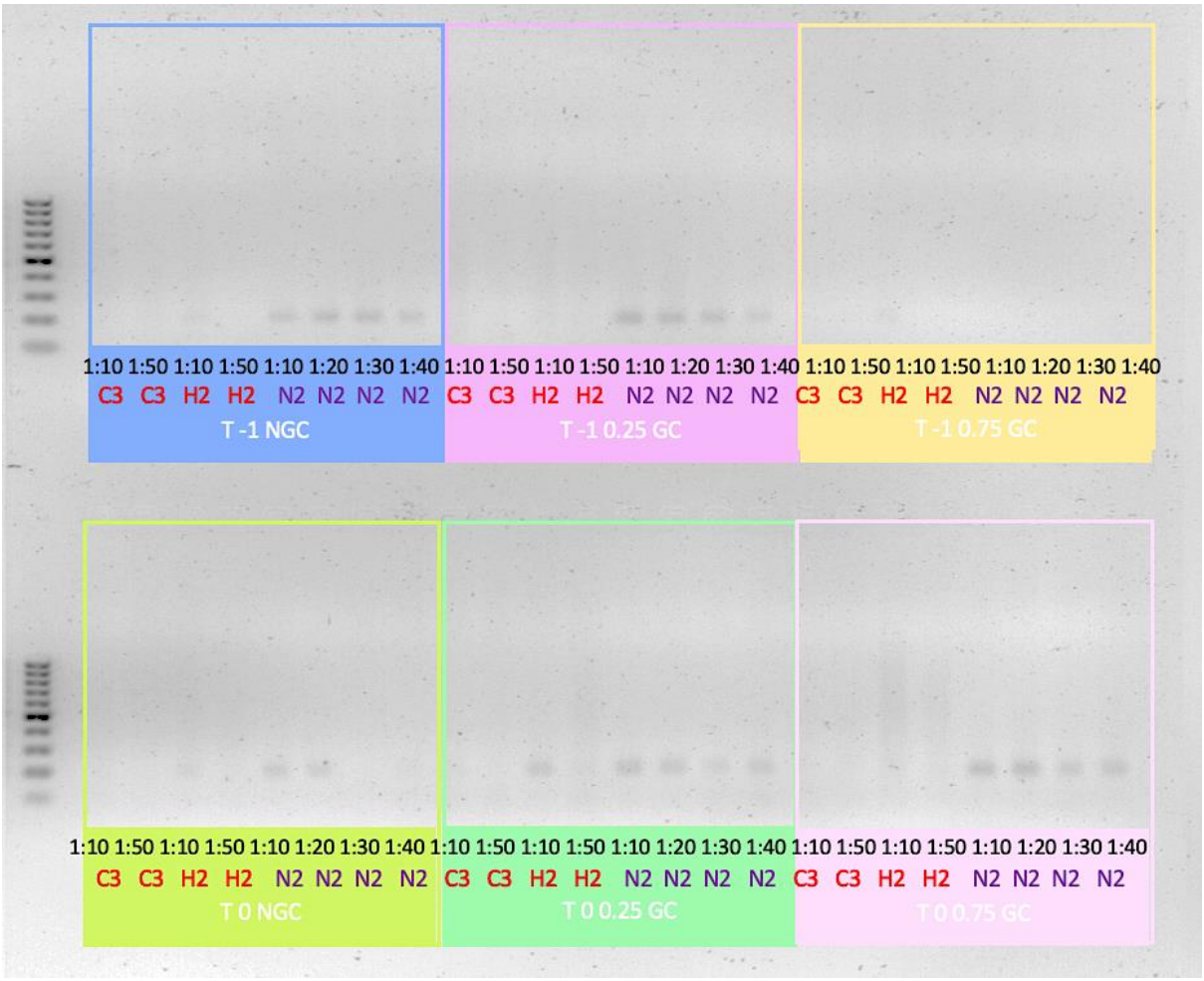

Figure B.3: Gels with PCR product of DNA extracted from *H. gammarus*, *H. americanus* and *N. norvegicus* testing the effect of annealing temperature and GC enhancer concentrations. PCR amplification using the initial annealing temperature (43°C) and 0.75 µl GC enhancer per reaction resulted in the best amplification of N. norvegicus while blocking the *Homarus* species.

Additional optimization experiments were carried out using DNA extractions from *H. gammarus*, *H. americanus* and *N. norvegicus*. The PCR plate was divided into three zones with different annealing temperatures: 0°C, -1°C, and -2°C lower than the initial annealing temperature (43°C). Each zone contained three strips with the same DNA templates but varying concentrations of GC Enhancer: 0 µl, 0.25 µl, and 0.75 µl per reaction. Three NTCs, one for each GC enhancer concentration, was included in the first temperature zone to control for contamination. Reaction mixture and thermocycling conditions were as described in the previous paragraph. The best results were obtained by using 0.75 µl GC enhancer and an annealing temperature of 43°C (Figure B.3). Pictures of the PCR product amplified at 41°C was omitted as no product was observed on the gel. The melt curve diagram from qPCR showed successful amplification of only *N. norvegicus* (Figure B.4).

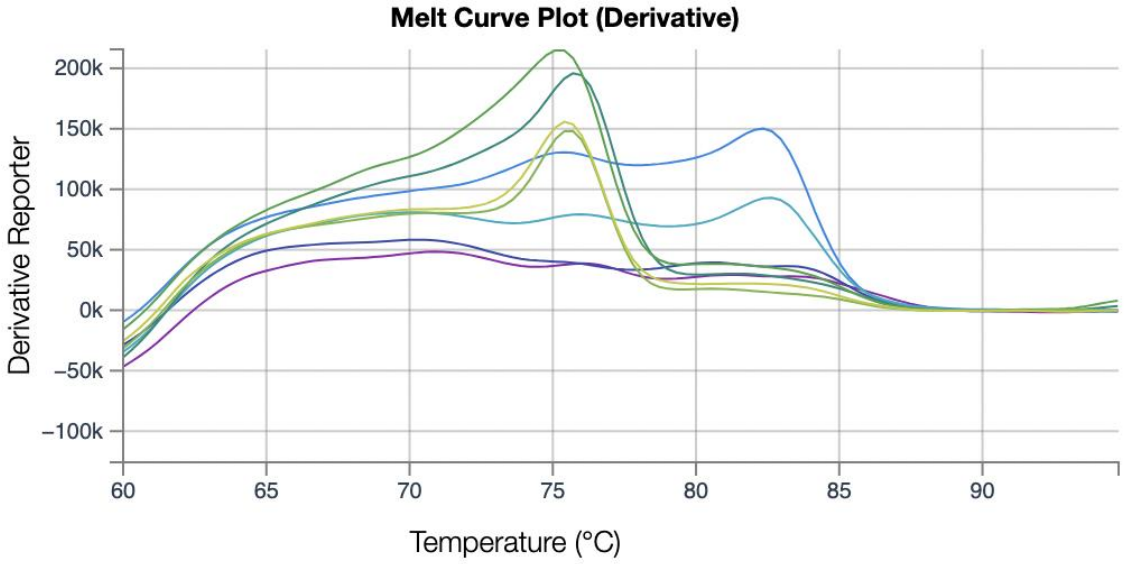

Figure B.4: Melt curve plot from qPCR of extracted DNA templates from *H. gammarus*, *H. americanus* and *N. norvegicus*. Peaks indicate a significant decrease in the fluorescent dye (Sybr Green) signal, meaning that there is double stranded DNA in the PCR product mix. The temperature value at the peak indicates the melting temperature of the double stranded PCR product. The four blue/purple curves show no successful amplification of the DNA from *Homarus* species, while the four green curves each have one distinct peak showing successful amplification of *N. norvegicus*.

Appendix C: Optimizing COI protocol after failed quality check

The PCR optimizing experiment involved 24 samples showing good amplification in earlier trials, with 0.3 µl BSA per sample, three annealing temperature levels: 44°C, 45°C, and 46°C, and three GC Enhancer concentration levels: 0.5 µl, 1 µl, and 1.5 µl per reaction. The optimal method was using an annealing temperature of 46°C and no GC enhancer. Gel electrophoresis results checking amplification of 200 bp fragments after bead cleaning using 0.8:1 bead to sample ratio (left picture) and using the optimized PCR method (right picture) is shown in Figure C.1. No significant amount of product (at 200 bp) was produced.

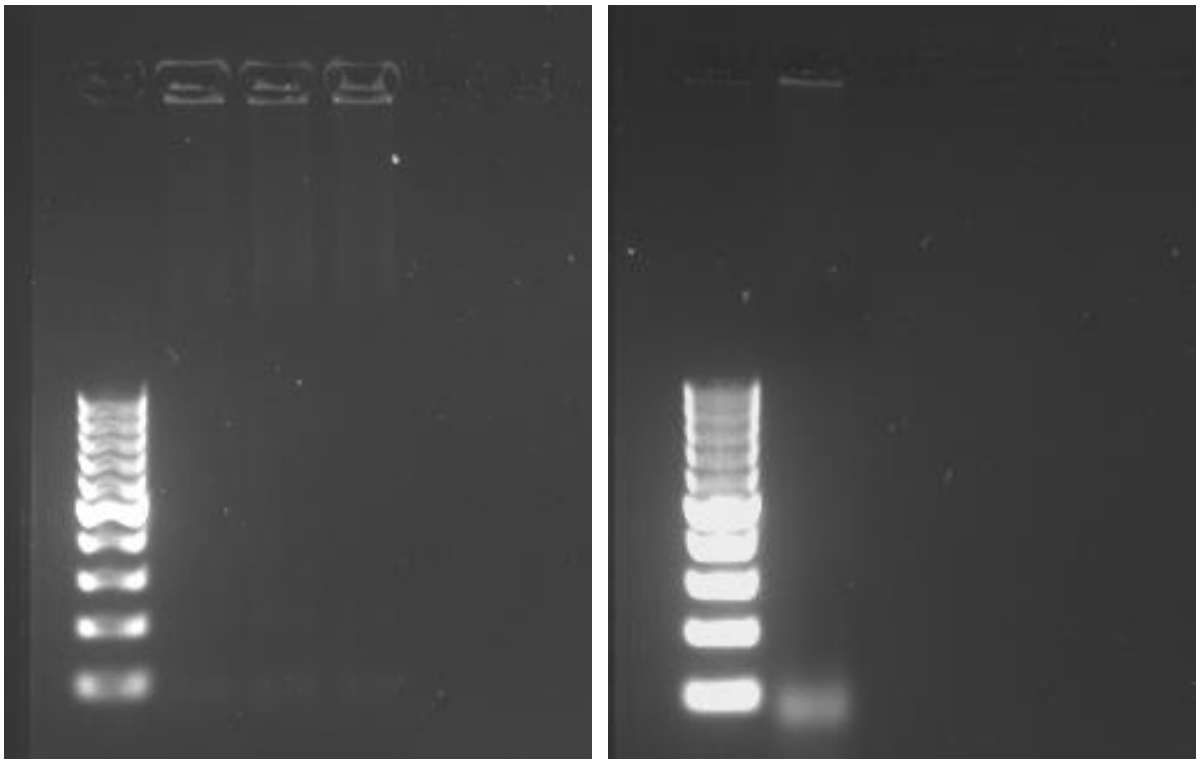

Figure C.1: Gel electrophoresis results from running pooled amplicons after 0.8:1 bead to sample ratio (left picture) and from using an optimized method (46°C, 0 µl GC, 0.3 µl BSA) (right picture) on samples in COI plate 1. The brightest band on both ladders (left side on each gel) marks a product size of 500 base pairs. The amplicon product on both gels is below the band marking 100 bp in size, and in a) the band containing amplicon pools are barely visible.

Appendix D: Grouping of soft-bodied, hard-bodied and hard-part containing organisms

Soft-bodied organisms, organisms with hard exoskeletons and organisms lacking a hard exoskeleton but having hard parts or tough tissue are showed in table D.1, D.2, and D.3, respectively.

Table D.1: List of phylum and designated group of soft-bodied taxa.

Phylum	Group	Taxa
Cnidaria	Coral animals	Anthozoa class, Actiniaria order
	Hydrozoans	Anthoathecata order, Leptothecata order
	Jellyfish (Scyphozoa)	Cyanea genus, Semaeostomeae order
	Myxozoans	Myxozoa class
Nematoda	Nematodes	Oncholaimidae family, Chromadoridae family, Anticomidae family, Enoplida order
Nemertea	Nemerteans	Heteronemertea order
Gastropoda	Nudibranchs	Onchidoris muricata
Chordata	Tunicates	Ascidiacea class, Phlebobranchia order, Oikopleura dioica, Molgula genus, Stolidobranchia order, Ascidiidae family, Molgulidae family

Table D.2: List of phylum and designated group of taxa with a hard exoskeleton.

Phylum	Group	Taxa
Mollusca	Mollusks	Lyonsia norwegica, Gastropoda class
Arthropoda	Arthropods	Hippolytidae family, Aegisthidae family, Amonardia coreana, Calanoida order, Centropages typicus, Cyclopina genus, Miraciidae family, Oithonidae family, Paracalanus genus, Harpacticoida order, Acartiidae family, Decapoda order, Hexanauplia class, Insecta class, Nephropidae family, Podocopida order
Echinodermata	Sea urchins	Echinoidea class

Table D.3: List of phylum and designated group of taxa lacking a hard exoskeleton but having hard parts or tough tissue.

Phylum	Group	Taxa
Bryozoa	Bryozoans	Cheilostomatida order, Triticella flava, Cyclostomatida order, Gymnolaemata class
Echinodermata	Echinoderms	Amphilepidida order, Apodida order, Asteroidea class
Chlorophyta	Green algae	Rhizoclonium genus, Trebouxiophyceae class, Ulotrichales order, Tetraselmis genus
Rhodophyta	Red algae	Bonnemaisonia genus, Ceramiaceae family, Erythrotrichiaceae family, Rhodomelaceae family
Streptophyta	Flowering plants	Pinopsida class, Magnoliopsida class, Malpighiales order

Chordata	Fish	Gadiformes order, Actinopteri class, Pleuronectiformes order
Haptophyta	Coccolithophorids	Noelaerhabdaceae family
Annelida	Polychaetes	Spionida order, Syllidia sp. A ZW-2021, Notomastus genus, Polychaeta class, Pectinariidae family
Porifera	Sponges	Demospongiae class

Appendix E: Data quality report results

The forward sequence reads were of poor quality and led to substantial data loss in the filtering step. Filtering the sequences with standard parameters dropped any sequence with an error rate above the expected error. An example is given in Figure E.1 and E.2 showing the quality score distribution and error rate distribution along reads for sample L20_17, respectively. The quality score of most positions along the sample reads were below 30, which is the benchmark quality score for Illumina platforms (Figure E.1). Multiple bases along the reads had base error rates over 0.3% (Figure E.2).

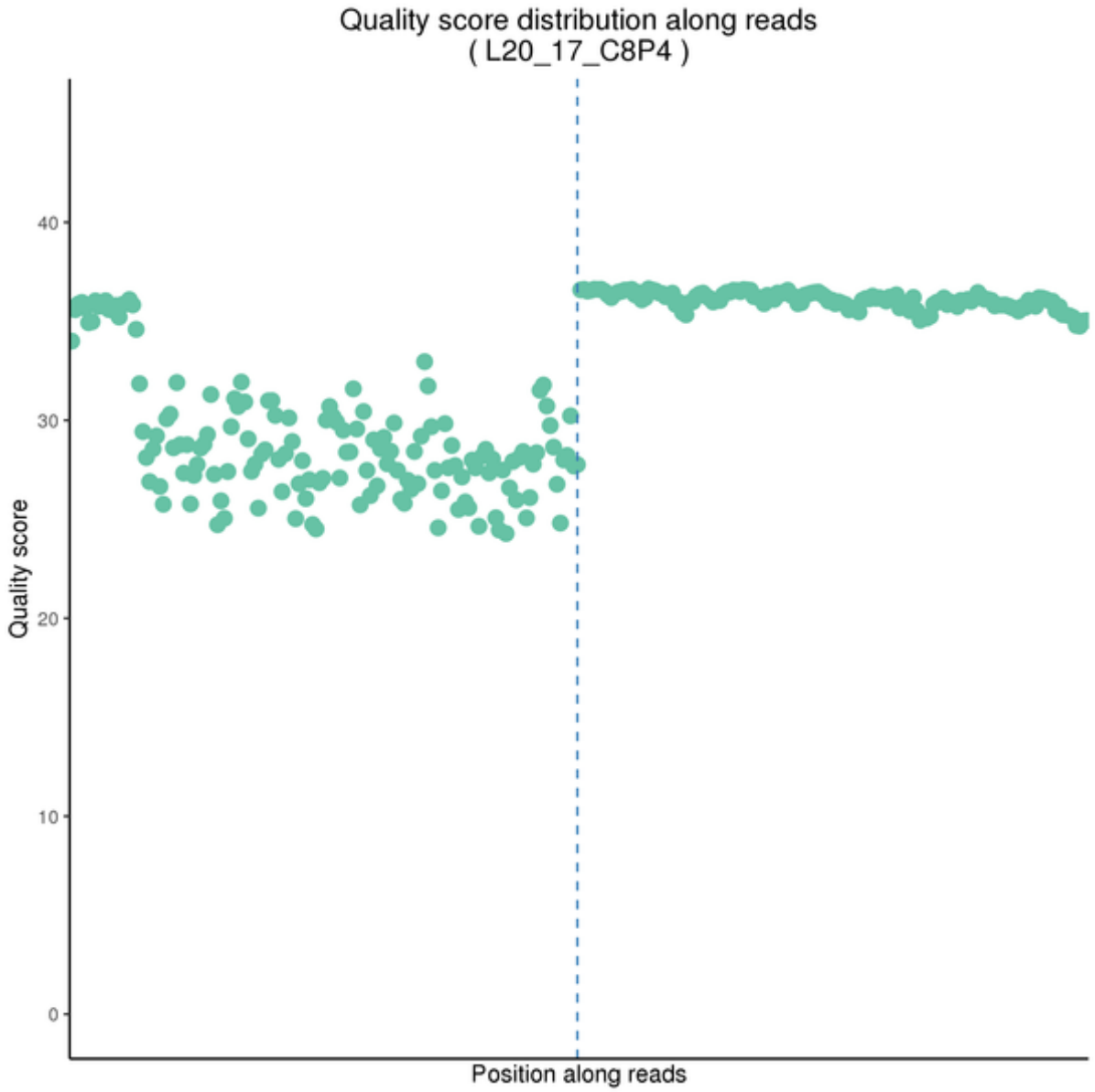

Figure E.1: Quality score distribution along sequence reads from sample L20_17. Retrieved from the quality control analysis report received from the sequencing provider (Illumina).

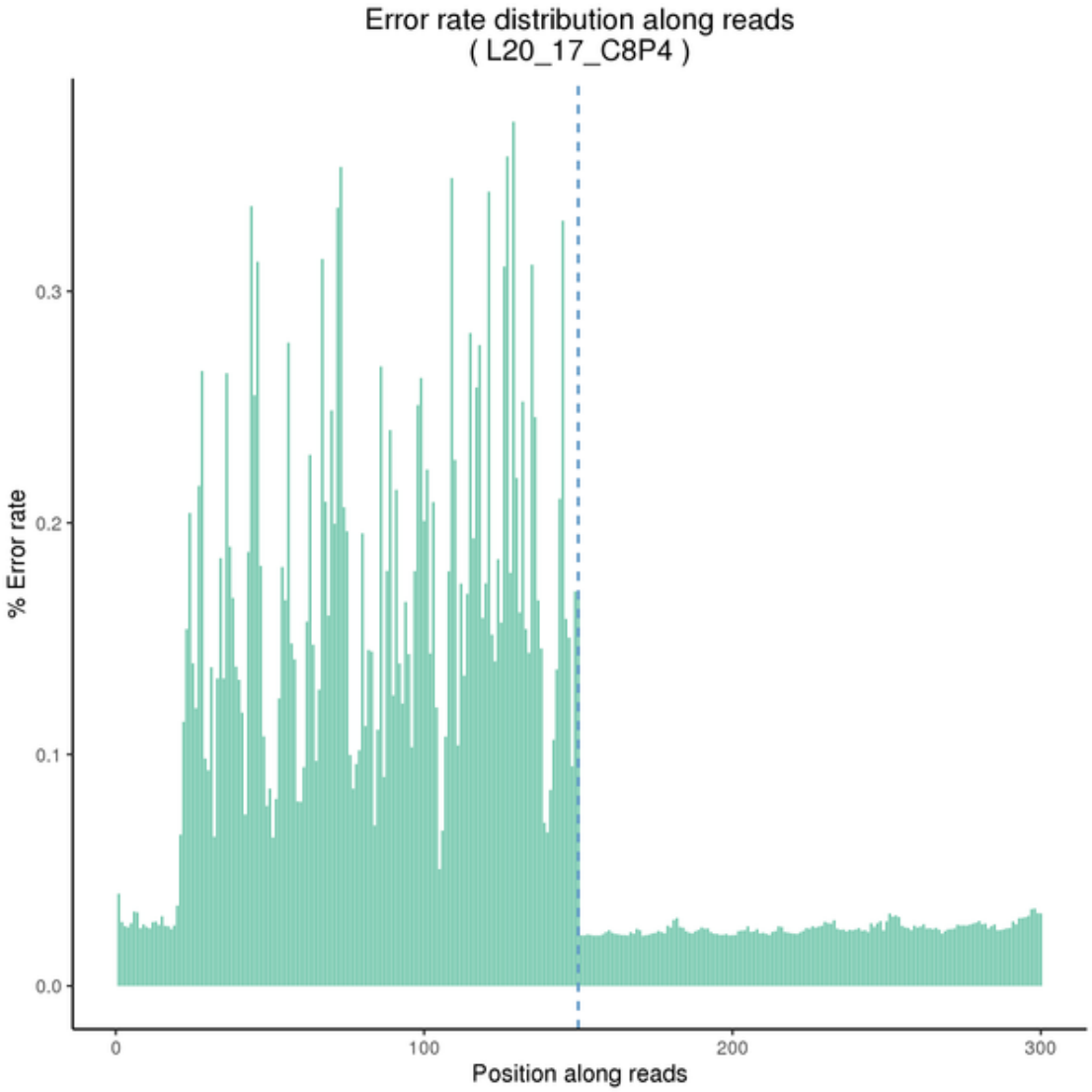

Figure E.2: Error rate distribution along sequence reads from sample L20_17. Retrieved from the quality control analysis report received from the sequencing provider (Illumina).

Appendix F: Phyloseq filtering parameters consequences

Figure F.1 illustrates an example of how the choice of filtering parameters influence the interpretation of the data, with different replicate thresholds and sequence depths influencing the average number of ASVs found per sample. Increasing sequencing depth decreases the number of samples which increases the number of ASVs per sample. Increasing the replicate filtering threshold decreases the number of ASVs which leads to a decrease in number of ASVs per sample.

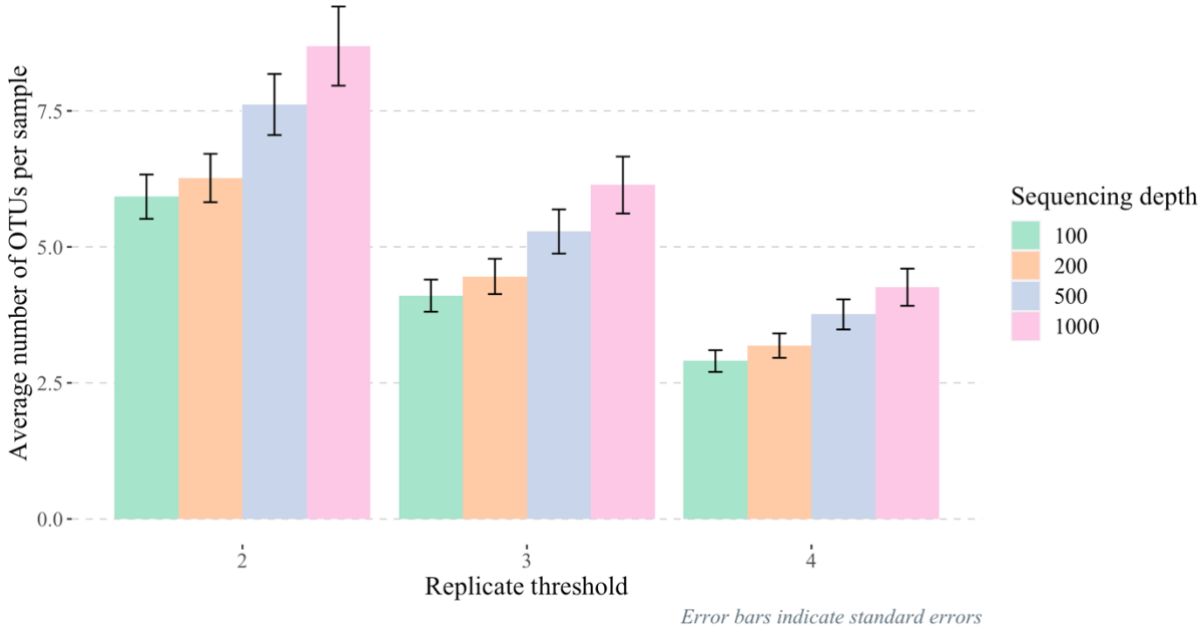

Figure F.1: Effect of sequencing depth and replicate filtering threshold on the average number of ASVs found per fecal sample. Error bars indicate the standard error of the averages. Filtering by sequencing depths 100, 200, 500, and 1000 kept samples with ≥ 100, 200, 500, or 1000 sequences. Filtering by replicate thresholds 2, 3, and 4 kept ASVs that were present in ≥ 2, 3, 4 PCR replicates.

NTNU

Norwegian University of
Science and Technology