Marine Environmental Research 191 (2023) 106162

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Supporting ecosystem services of habitat and biodiversity in temperate seaweed (*Saccharina* spp.) farms

Emilly Schutt[a], Rene Francolini[b,c], Nichole Price[b], Zachary Olson[d], Carrie J. Byron[a,*]

[a] School of Marine and Environmental Programs, University of New England, 11 Hills Beach Road, Biddeford, ME, 04005, USA
[b] Bigelow Laboratory for Ocean Sciences, 60 Bigelow Drive, East Boothbay, ME, 04544, USA
[c] School of Marine Sciences, University of Maine, Darling Marine Center, 193 Clarks Cove Road, Walpole, ME, 04573, USA
[d] School of Social and Behavioral Sciences, University of New England, 11 Hills Beach Road, Biddeford, ME, 04005, USA

A R T I C L E I N F O

Keywords:
Ecosystem services
Aquaculture
Seaweed
Macroalgae
Kelp
eDNA
GoPro
Camera
Invertebrate
Fish

A B S T R A C T

Habitat provisioning, and the biodiversity within, is considered a type of “supporting” ecosystem service. Ecosystem services are the benefits humans receive from healthy ecosystems. We assess whether kelp (*Saccharina* spp.) farms provide seasonal habitat for wild organisms. Contrary to other studies conducted in tropic seaweed farms, we did not observe habitat provisioning or increased biodiversity at seasonal temperate seaweed farm sites compared to neighboring non-farm sites, which is encouraging news for the aquaculture industry given that most farm gear is removed from the water after the spring harvest. We quantified fish and crustaceans interacting with kelp farms using GoPro cameras. We also assessed small (<5 mm) invertebrates using mesh settling devices suspended at the same depth as kelp lines (2m). Visual surveys were paired with eDNA. There was coherence in the conclusions drawn from observational and eDNA methods, despite weak coherence in the specific species identified between the methods. Both farm and non-farm sites exhibited higher species richness and biodiversity in the summer non-growing season compared to the winter growing season, attributed to expected seasonal species movements.

1. Introduction

The seaweed industry is rapidly growing in the United States, though a majority of global seaweeds are produced and consumed in Asian countries, primarily China (FAO, 2022). Over the last decade, improved cultivation methods have enabled the industry to grow rapidly in the US, with the state of Maine leading innovation in cultivation techniques and production of biomass (Flavin et al., 2013; Kim et al., 2019; McKinley Research Group, L, 2021; Piconi, 2020). Maine’s algae harvest has increased from 6614 wet kg in 2015 to 226,796 wet kg in 2020 (MEDMR, 2020). With the continued projected growth of the industry (Piconi, 2020), it is important to analyze how the addition of farm structure and kelp biomass is interacting with wild ecosystems. One way to examine kelp farm interactions with, or contributions to, existing ecosystems is through the lens of ecosystem services.

Ocean and coastal ecosystems provide important ecosystem services valued at billions of dollars each year (Beaumont et al., 2008; TEEB, 2012). Ecosystem services are material and non-material benefits humans obtain at no cost from a healthy ecosystem (Daily, 1997; Westman, 1977). Ecosystem services provided by animal aquaculture (finfish, bivalves, crustaceans), have been studied more extensively compared to seaweed aquaculture (Weitzman, 2019). Ecosystem services are often categorized into four groups: provisioning services (e.g. providing food and products), regulating services (e.g. nutrient cycling), cultural services (e.g. sense of place) and supporting (e.g. habitat provisioning) (Barrett et al., 2022; Duarte et al., 2021; Gentry et al., 2020). Provisioning services describe products that can be sold in the marketplace from the harvested seaweed. Regulating services of seaweed aquaculture are currently being heavily studied, particularly how farms can play a role in carbon cycling (Fujita et al., 2023; Krause-Jensen et al., 2018; Krause-Jensen and Duarte, 2016; Ricart et al., 2022). Cultural services recognize tacit values and human connection to an environment towards a sense of wellbeing or livelihood. Supporting services of seaweed aquaculture, including habitat creation, are the least studied type of ecosystem service (Weitzman, 2019).

The limited amount of research that has been conducted on seaweed aquaculture supporting services has focused on habitat creation (Bekkby et al., 2023; Corrigan et al., 2022; Theuerkauf et al., 2022). However,

* Corresponding author.
E-mail address: cbyron@une.edu (C.J. Byron).

https://doi.org/10.1016/j.marenvres.2023.106162
Received 20 April 2023; Received in revised form 16 August 2023; Accepted 30 August 2023
Available online 11 September 2023
0141-1136/© 2023 Published by Elsevier Ltd.

the majority of these studies were conducted in tropical climate zones (Anyango et al., 2017; de Carvalho et al., 2017; Eklöf et al., 2006; Hehre and Meeuwig, 2015; Theuerkauf et al., 2022). In temperate regions, numerous studies list habitat creation as a potential ecosystem service of seaweed aquaculture (Gentry et al., 2020; Hasselström et al., 2018; Préat et al., 2018) but direct observations and measurements of biodiversity have demonstrated that kelp farms harbor fewer individuals and less taxa than wild kelp beds and do not provide equivalent habitat (Bekkby et al., 2023; Forbes et al., 2022)

Seaweed is grown on suspended ropes near the water surface (2 m depth), decoupling it from the benthos. Many finfish and large crustaceans common in the Gulf of Maine are bottom-associated organisms, including; American lobster (*Homarus americanus*), Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic sturgeon (*Acipenser oxyrhynchus oxyrhynchus*), black sea bass (*Centropristis striata*), cusk (*Brosme brosme*), haddock (*Melanogrammus aeglefinus*), tautog (*Tautoga onitis*), thorny skate (*Abrlyraja radiata*), and winter skate (*Leucoraja ocellata*) (Collette and Klein-Macphee, 2002). It can be assumed these organisms would have little to no interaction with the biomass of the kelp farm because they live and feed near or on the benthic substrate and several of them migrate offshore during the winter when farms are suspended in nearshore waters (Collette and Klein-Macphee, 2002). Structural architecture of how the kelp biomass grows on the farm could also affect smaller invertebrates that use wild kelp holdfasts, stipes, and blades as microhabitats and influence trophic interactions (Teagle et al., 2017; Walls et al., 2016). In a kelp farm, these microhabitats are near the water's surface displacing colonizing invertebrates from benthic predators (Teagle et al., 2017).

The goal of this research is to assess habitat services of kelp farms, as compared to analogous non-farm sites, in the Gulf of Maine. As the kelp farming industry in Maine continues to grow, it is important to assess how kelp farms impact the environment and ecosystems around them (Visch et al., 2020), in comparison to these same areas if they otherwise had no operating farms present. Although this novel study is site-specific, factors such as kelp species cultivated, type of cultivation gear, and local environmental characteristics, could vary across farms within a geological region and change a farm's habitat value (Alleway et al., 2019; Theuerkauf et al., 2022). The value of marine habitat is often viewed as its ability to support a fishery resource and is often quantified using biomass or abundance data (Barrett et al., 2022; Theuerkauf et al., 2022).

In this study, we compare traditional observational methods with environmental DNA (eDNA) methods to determine if there is coherence between observational and genetic methods when assessing community composition in temperate kelp farms. eDNA can be extracted from environmental samples such as soil and water (Taberlet et al., 2012). eDNA methods rely on capturing skin cells, feces, gametes, or any other cellular or extracellular DNA that has dissociated from the organism (Monuki et al., 2021; Turner et al., 2014). More recently, eDNA methods have integrated into ecological studies and are now used as a biomonitoring tool (Taberlet et al., 2012). Due to the novelty of this technique in ecology, few comparative studies between eDNA and traditional community surveying methods have been done (Lamy et al., 2021; Liu et al., 2019; Port et al., 2016; Stoeckle et al., 2021; Thomsen et al., 2012). Though, it has been demonstrated that eDNA not only detects species seen during underwater visual census, it outperforms underwater visual census by detecting rare and cryptic species (Lamy et al., 2021; Port et al., 2016). Interestingly, eDNA can detect differences in vertical and horizontal spatial capacities within kelp forests (Monuki et al., 2021). Given these findings, the relative cost-effectiveness, and the recommendation of using eDNA to assess habitat services of seaweed aquaculture (Corrigan et al., 2022; Taberlet et al., 2012; Thomsen and Willerslev, 2015), there is potential for eDNA to replace traditional survey methods for community analysis in, and out of, seaweed farms.

The aims of this research were to; (1) determine the alpha and beta diversity of finfish and large crustaceans in kelp farms versus unfarmed sites using visual surveys; (2) determine the alpha and beta diversity of small invertebrates in kelp farms versus unfarmed sites using visual surveys; and (3) to verify whether conclusions drawn from eDNA methods for assessing biodiversity align with those from visual assessments of biodiversity. Due to structural decoupling of cultivated kelp from the benthos, Maine's benthic fish community assemblages, and the seasonality of kelp farming, we hypothesized there may not be strong differences in alpha or beta diversity between kelp farm and its non-farm reference areas, despite documentation of seaweed farms providing habitat in other regions (Alleway et al., 2019; Corrigan et al., 2022; Gentry et al., 2020; Theuerkauf et al., 2022; Walls et al., 2016).

2. Methods

Since this study aimed to provide a starting point on the influence of kelp farming on surrounding organism communities in the Gulf of Maine, we quantified species richness (alpha diversity), biodiversity (alpha diversity), as well as determine the specific organisms driving changes between particular explanatory variables (beta diversity) using observational methods of GoPro camera visual surveys and pelagic small invertebrate collections. Environmental DNA (eDNA) methods were also incorporated to determine whether genetic data corroborate with observational data.

2.1. Study sites

This research was conducted on four licensed aquaculture sites in the Gulf of Maine with two leases each in Saco and Casco bays (MEDMR, 2022) (Fig. 1). In Saco Bay, both farms were mono-culture experimental leases growing *Saccharina latissima* (sugar kelp) and/or highly related *S. angustissima* (skinny kelp) (Augyte et al., 2019). These farms were located off the northeast coast of Ram Island and the southern coast of Wood Island. The Ram Island farm (lease: SACO RIx; −70.351666, 43.468467) resided in 13–17m of water at high tide and consisted of a total horizontal grow line length and total harvested biomass of 354m and 1,700 kg in 2021, and 560m and 1,830 kg 2022. The Wood Island farm (license: CBYR121; −70.337042, 43.455984) resided in 6 – 9m at high tide with a total horizontal grow line length and total harvested biomass of 46m and 250 kg in 2021, and 60m and 300 kg in 2022. In Casco Bay, the farms were standard leases located off the southwest coast of Great Chebeague Island (lease: CAS CHEB2; −70.146710, 43.723977) and off the southeast coast of Clapboard Island (lease: CAS CF3; −70.191074, 43.715364) and both grew skinny kelp. The lease near Great Chebeague Island was a mono-culture lease residing in 9–12m of water at high tide with a total horizontal grow line length of 3, 657m and total harvested biomass of 23,975 kg in 2021 and 21,782 kg in 2022. Finally, the lease near Clapboard Island was a co-culture farm growing kelp and blue mussels (*Mytilus edulis*) residing in 12–15m of water at high tide with a total horizontal kelp grow line length of 4, 572m and harvested biomass of 21,905 kg in 2021 and 17,416 kg in 2022. The mussels were grown on 42,120m of vertical mussel ropes hanging from 9 of the 12 rafts present, all with dimensions of 12m by 12m.

Each aquaculture farm plus a 100m radius around it will represent a "site" and will be referred to by the island it resides near. Within each site, two separate areas were designated, a farm area and a non-farm area. The farm area was near (≤5m) the outer grow line, and the non-farm area was approximately 100m away from the kelp farm. Preliminary evaluations demonstrated a change in water biochemistry and community composition at this distance (Groves, 2019). The paired farm and non-farm areas of a site were approximately the same depth (±1.5m) and had the same type of soft benthic substrate. Data was collected year-round from November 2020 through August 2022. Seasonality of the winter growing season compared to the summer non-growing season was determined by the timing of deployment and removal of the kelp farm. The winter growing seasons (farm structure

Fig. 1. Study sites in southern Maine. Clapboard Island farm (A) and Chebeague Island farm (B) are located in Casco Bay. Ram Island farm (C) and Wood Island farm (D) are located in Saco Bay.

and kelp biomass deployed) occurred from November 2020 to May 2021 and from December 2021 to May 2022. The summer non-growing season, with farm structure removed and kelp biomass harvested, occurred from June 2021 to November 2021 and June 2022 to August 2022.

2.2. Camera visual surveys

GoPro cameras (Hero 6 and Fusion 360) were deployed to observe large mobile finfish and crustaceans that may have been within our sites. Each site was visited once a month during the mid-morning daytime hours. Due to access limitations, sites in Saco Bay were primarily visited near low tide while sites in Casco Bay were visited near high tide. During the winter growing seasons at farm areas, three cameras were deployed at two different camera positions: one surface camera and two benthic cameras. In the farm area, a surface camera was attached to a farm spacer buoy and a benthic camera was lowered onto the seafloor. In the non-farm area, only a benthic camera was deployed due to the absence of farm structure. Using this experimental design, we compare benthic observations between a farm area and its reference. We also compare surface and benthic observations within farm areas. During the summer non-growing season, only benthic cameras in farm and non-farm areas were deployed, due to the absence of farm structure to hold and stabilize a surface camera. For both the winter growing and summer non-growing seasons, the cameras were deployed to record activity for approximately 2 h on time lapse mode with an interval setting of 0.5sec. Camera footage was watched using GoPro Player software and findings were recorded. Each camera deployed was deemed one datum point for the length of its drop duration.

2.3. Small invertebrate collections

Invertebrates were collected using round plastic kitchen scrubber sponges measuring 8 cm in diameter (White et al., 2019) that were housed in a large mesh bag to maximize waterflow through the sponges and suspended at 2m below the surface of the water at the depth of the kelp grow line. The mesh bag containing the sponges was attached to a buoy – rope – anchor system and is hereafter referred to as the collector device. Three sponges were used in each collector device, providing the necessary surface area to collect a representative sample at times of low biodiversity. Four invertebrate collector devices were independently anchored at every site, with two collector devices in each of the farm areas and two collector devices at each of the non-farm areas in order to collect small invertebrates passing through the sites at the 2m depth of the farmed kelp. The collector devices, that held the sponges, were kept clean of biofouling throughout the sampling periods to maximize waterflow and transport of small invertebrates to the sponges.

During the 2021 and 2022 winter growing seasons, invertebrates were collected during two peak periods of the kelp life cycle. The first collection sampling period was during the kelp's peak productivity (mid-February to late March) and the second sampling period was when the kelp reached its peak biomass (early April to mid-May) (Grebe et al., 2021). In addition, to be able to compare the invertebrate community during the winter growing season to the summer non-growing season, invertebrate collections were made at two additional sampling periods during early summer (mid-June to mid-July) and during late summer (mid-July to mid-August). These four time periods each lasted about 4-weeks and were repeated twice during the 2-year study (2020–2022). At the end of a sampling period, the sponges were brought back to the lab and stored in a −20 °C freezer until analysis. To dislodge invertebrates from the porous sponges, the sponges were thawed, cut, teased apart, and flushed with filtered seawater using sterile techniques. The invertebrates were identified to the lowest possible taxonomical level (genus or species), counted, and recorded.

2.4. Environmental DNA

Environmental DNA was collected in tandem with both camera visual surveys and small invertebrate collections in Saco Bay in 2021 to be able to compare species detections across visual surveys and eDNA methods. A targeted subset of samples, enough to address our objective

on whether eDNA methods corroborate with visual methods, were utilized. eDNA water samples were collected adjacent to camera deployments on a subset of deployment dates (March 23rd, April 15th, May 7th, June 9th, September 14th, and October 14th). Water was collected near benthic cameras on all 6 dates and near surface cameras on the 3 dates in March, April and May to correspond to the time when the farm was deployed. eDNA water samples were also collected next to surface invertebrate collector devices on the four dates when they were retrieved for observation: April 7th to correspond to the cultivated seaweeds peak productivity, May 13th to correspond to the cultivated seaweeds peak biomass, July 22nd to correspond to early summer period and August 27th to correspond to late summer period. On days when the sponges were retrieved from the small invertebrate collection devices, eDNA water samples were collected near and at the same depth of each invertebrate collector device. Every eDNA water sample was collected with a 5L Niskin, 1L of which was transferred into a bottle for transportation. In between each sample, the Niskin was cleaned using a ten percent bleach solution and three deionized water rinses. A negative field control of sterile DI water was rinsed in the sterile Niskin and collected to test our decontamination protocol for effectiveness. Samples were transported in a cooler on ice to limit DNA degradation until the water could be filtered which took place within 12hr of collection. Water samples were filtered onto a Whatman nitrocellulose membrane filter with pore size 0.2 μm and diameter 47 mm using sterile filter funnels and a vacuum pump. Filters were rolled and put into labeled 5 mL centrifuge tubes and stored in a −80 °C freezer until DNA extraction.

DNA was extracted using the Qiagen DNeasy PowerSoil Pro Kit following the manufacturers protocol other than the first step, where the beads were poured into the 5 mL tube containing the filter for the initial bead-beating step instead of using the provided 2 mL tube. Each sample was eluted in a final volume of 70 μL and stored at −20 °C. Total DNA concentration was quantified using a Qubit 2.0 Fluorometer (Life Technologies) and the Qubit dsDNA HS 1× assay. All samples were sent to Integrated Microbiome Resource (IMR) at Dalhousie University for amplicon sequencing, as described below.

Sixteen samples, from water collections coordinated with the camera deployments, were amplified using the universal 12S MiFish primers (Miya et al., 2015). Prior to shipping to IMR, these samples were PCR amplified in triplicate and cleaned in house to remove erroneous bacterial amplification that occurs with these primers. A touchdown PCR protocol was conducted using the Platinum superFi II Mastermix (Invitrogen) and the MiFish primers, with an initial activation at 95 °C for 2min, then 13 cycles of denaturing at 94 °C for 30sec, annealing at 69.5 °C (−1.5 °C/cycle) for 30sec and extension at 72 °C for 30sec, followed by 25 cycles of denaturing at 94 °C for 15sec, annealing at 50 °C for 20sec, and extension at 72 °C for 20sec, a final extension at 72 °C for 5min and holding at 12 °C forever. PCR products were then visualized on a 1.2% agarose gel and cleaned using the Select-a-Size DNA Clean & Concentrator MagBead Kit (Zymo Research), following the manufacturer's protocol and quantified via Qubit. These amplicons were then sent to IMR for sequencing.

A different set of sixteen samples, from water collections coordinated with the small invertebrate collectors, were amplified in triplicate using the universal 18S primers (Stoeck et al., 2010). These targeting the small subunit RNA gene region. These samples did not need to be pre-amplified and were sent as pure genomic DNA extracts. IMR carried out library preparation for all samples and sequenced them on an Illumina MiSeq platform (2 × 150 paired-end sequencing for the 12S samples and 2 × 300 paired-end sequencing for the 18S samples).

Bioinformatic analyses were conducted using the Maine-eDNA dada2 pipeline (Tupper, 2023a, 2023b; Tupper & Sleith, 2023a, 2023b). In short, this pipeline uses cutadapt (2.10) to remove primers and trim reads, followed by DADA2 (4.2.2) to quality filter sequence reads, generate an error model, identify amplicon sequence variants (ASVs), and assign taxonomy. The 12S MiFish samples were analyzed using the default parameters as defined on github, except for parameter TruncLen, which was set to (0,0) to account for the short (170bp) amplicon length expected for this primer set. Taxonomic assignments were completed using a hand-curated 12S database consisting of finfish species found in the Gulf of Maine region. The 18S samples were analyzed using all default parameters as defined on github, and taxonomic assignments were completed using the PR2 database (https://pr2-database.org/). For all samples, R package PHYLOSEQ was then used to filter ASVs to retain only those that occurred at least 50 times across all samples. Furthermore, ASVs that occurred in only one of the three triplicates for each sample were removed, as well as ASVs that were singletons. At this point, statistical analysis and data visualization were carried out on the resulting dataset.

2.5. *Statistical analysis*

All statistical analyses were completed using R software, version 4.1.2 and significant statistical differences were determined at $\alpha = 0.05$. Six categorical variables (bay, site, area, camera position, season, time period) were used to determine potential differences in alpha and beta diversity measurements (Table 1).

For camera visual surveys, species richness was calculated as the total number of species observed during one camera's entire deployment. Species richness for small invertebrate collections was considered the total number of species seen across small invertebrate collection devices. Biodiversity was calculated for camera visual surveys and small invertebrate collections using the Shannon diversity index, $H = -\Sigma(\rho_i * \ln(\rho_i))$ (Shannon, 1948), where $\rho_i = (n_i/N)$; the number of individuals for species ($i = n_i$) and total number of individuals across all species (N). Normality and equal variance assumptions for parametric tests were conducted prior to univariate analysis using Shapiro-Wilks and Levene's tests. If the response variables did not meet either assumption, then the data were rank transformed (Conover and Iman, 1981). To determine differences in alpha diversity measurements, a nested analysis of variance (ANOVA) was used since not all variables were independent of each other. Data for alpha diversity analysis on camera visual surveys only included benthic cameras, as those cameras were consistently deployed through the entire study. To explore potential differences between camera positions, a separate dataset containing only cameras deployed in the farm areas, during the growing season was used to complete a *t*-test.

For beta diversity, datasets for camera visual surveys and small invertebrate collections were organized in two ways: by presence/absence, and by relative abundance. Dissimilarities were calculated for data organized as presence/absence (1- Sørensen index) (Anderson et al., 2011) and permutation analysis of variance (PERMANOVA) tests were used to determine beta diversity differences. Negative binomial

Table 1
Description of each categorical variable used in statistical analyses assessing species diversity.

Categorical Variables	Factors	Description
Bay	Casco Bay (CB), Saco Bay (SB)	2 bays
Site	Chebeague, Clapboard, Ram, Wood	4 sites (Chebeague & Clapboard in CB; Ram & Wood in SB)
Area	farm, non-farm reference	Paired farm area and non-farm area within each site
Camera position	benthic, surface	Surface cameras deployed on farms during winter growing season only
Season	Winter growing season, Summer non-growing season	Farming activity is inherently coupled with season
Time Period	peak productivity, peak biomass, early summer, late summer	4 periods each 4-weeks long during which small pelagic invertebrates were collected

modeling was used on data organized by relative abundance to determine beta diversity differences.

Non-metric multi-dimensional scaling (NMDS) plots were created using eDNA data to determine potential organism community differences between explanatory variables. The NMDS plots were examined for clustering, which indicates differences in organism communities seen between explanatory variable factors. Whether clustering was present or absent, was compared to whether significant differences were found for observational univariate statistics.

3. Results

3.1. Diversity characterization

Across all observations, the camera visual surveys revealed 16 finfish, large crustacean, and mammal species (Table 2). Typically, 1–2 species were seen per camera deployment with an overall range between 0 and 7 species per deployment. All deployments occurred during daylight hours with 83.7% of the camera deployments occurring between 8:00–13:00 eastern standard time. These findings were made from a total of 160 camera deployments each with a duration averaging 2.11 SD $\pm$0.30 h (range: 1.75–2.75) and conducted between November 2020 and August 2022 capturing a total of 338 h of footage across all deployments. The duration of deployment did not affect the species richness or biodiversity observed (Fig. S1). Beta diversity was analyzed according to both species' presence and abundance. The species seen most often on cameras was the Atlantic rock crab (*Cancer irroratus*) with 59 individual sightings. Jonah crabs (*Cancer borealis*) were the second most sighted (54 times) and American lobsters (*Homarus americanus*) were sighted 42 times. Average dissimilarities using 1- Sørensen index were 0.69 (range 0–1) for camera visual surveys. The PERMANOVA and negative binomial modeling from the camera visual surveys suggests the same species are driving these differences (Table 3).

The small invertebrate collections revealed 15 species (Table 4). The amphipod (*Lembos websteri*) comprised of 34.8% of total small invertebrates caught. The second most abundant species caught across all collections was the copepod (*Paracalanus* sp.) (24.7%) and the third most abundant was the skeleton shrimp (*Caprella linearis*) (20.4%). These findings were made from a total of 88 invertebrate collection devices that were analyzed across the four sampling time periods: peak seaweed productivity, peak seaweed biomass, early summer, and late summer, each lasting about 4 weeks. Average dissimilarities using 1-Sørensen index were 0.55 (range 0–1) for small invertebrate collections leading to different conclusions from these two analyses (Table 3). Specifically, the Permanova result suggests a difference in the presence/absence of species between seasons and between time periods whereas the diversity negative binomial modeling of abundance suggests that there is also a weak difference in diversity between bays and between sites (Table 3).

The eDNA MiFish database for finfish revealed 43 species. These findings were made from a total of 16 independent water samples taken in Saco Bay on dates March 23rd, April 15th, May 7th, June 9th, September 14th, and October 14th in 2021 (Table 2). The MiFish database is specific to finfish and does not include crustaceans. It was the only sequencing database used on benthic water samples to correspond with benthic camera deployments where finfish were expected to be observed.

The eDNA 18S sequencing and PR2 database revealed 411 species. A total of 16 independent water samples were taken in Saco Bay on dates April 7th, May 13th, July 22nd, and August 27th in 2021 (Table 4). When looking at species detections, we saw 7 joint presences between eDNA and camera visual surveys, 7 species were present on cameras but not eDNA and 21 occurrences of organisms were present in eDNA but not on a camera (Table 2). For small invertebrate collections, we saw 4 joint presences between eDNA and small invertebrate collectors, 35 occurrences of organisms present in collectors but absent in the eDNA and 2 occurrences of organisms present in eDNA but not in collectors (Table 4).

Table 2
The species list represents all species observed on cameras at any of the locations throughout the duration of the study (2020–2022). Water samples for eDNA analysis were collected on these 6 dates in 2021 in Saco Bay while the cameras were deployed. Organisms detected by 12S eDNA sequencing and the MiFish database are shaded grey, and organisms observed on camera on that same date are noted with an "X". There were additional species identified by the MiFish eDNA database that are not listed here.

<table>
<tr><th>Species</th><th>Mar 23</th><th>Apr 15</th><th>May 07</th><th>Jun 09</th><th>Sep 14</th><th>Oct 14</th></tr>
<tr><td>Acipenser oxyrinchus (Atlantic sturgeon)</td><td></td><td></td><td></td><td>X</td><td bgcolor="grey">X</td><td bgcolor="grey"></td></tr>
<tr><td>Brevoortia tyrannus (Atlantic menhaden)</td><td></td><td></td><td bgcolor="grey"></td><td bgcolor="grey">X</td><td bgcolor="grey"></td><td bgcolor="grey"></td></tr>
<tr><td>Clupea harengus (Atlantic herring)</td><td></td><td bgcolor="grey"></td><td bgcolor="grey"></td><td bgcolor="grey"></td><td bgcolor="grey"></td><td bgcolor="grey">X</td></tr>
<tr><td>Cyclopterus lumpus (lumpfish)</td><td></td><td>X</td><td>X</td><td></td><td></td><td></td></tr>
<tr><td>Hemitripterus americanus (sea raven)</td><td></td><td>X</td><td></td><td bgcolor="grey"></td><td></td><td bgcolor="grey"></td></tr>
<tr><td>Morone saxatilis (striped bass)</td><td></td><td></td><td bgcolor="grey"></td><td></td><td bgcolor="grey"></td><td bgcolor="grey">X</td></tr>
<tr><td>Myoxocephalus Scorpius (shorthorn sculpin)</td><td></td><td></td><td bgcolor="grey">X</td><td></td><td></td><td bgcolor="grey"></td></tr>
<tr><td>Tautogolabrus adspersus (cunner)</td><td></td><td></td><td bgcolor="grey"></td><td bgcolor="grey"></td><td bgcolor="grey">X</td><td bgcolor="grey"></td></tr>
<tr><td>Pholis gunnellus (rock gunnel)</td><td bgcolor="grey"></td><td bgcolor="grey"></td><td bgcolor="grey"></td><td bgcolor="grey"></td><td bgcolor="grey"></td><td bgcolor="grey">X</td></tr>
<tr><td>Pseudopleuronectes americanus (winter flounder)</td><td></td><td></td><td></td><td>X</td><td></td><td>X</td></tr>
<tr><td>Leucoraja erinacea (little skate)</td><td></td><td></td><td></td><td></td><td>X</td><td></td></tr>
<tr><td>Cancer borealis (Jonah crab)</td><td colspan="6" rowspan="6">No crustaceans or mammals included in the 12S primer and MiFish database</td></tr>
<tr><td>Cancer irroratus (Atlantic rock crab)</td></tr>
<tr><td>Carcinus maenas (green crab)</td></tr>
<tr><td>Homarus americanus (American lobster)</td></tr>
<tr><td>Poca vitulina (harbor seal)</td></tr>
<tr><td>Phocoena phocoena (harbor porpoise)</td></tr>
</table>

Table 3
Results from camera visual surveys and small invertebrate collections multivariate statistical analysis, PERMANOVA (presence/absence) and negative binomial modeling (relative abundance) between seasons (winter growing, summer non-growing), bays (Saco, Casco), sites (Ram, Wood, Clapboard, Chebeague), areas (farm, non-farm), camera position (surface, benthic) and time period (peak productivity, peak biomass, early summer, late summer) α = 0.05.

	df	*F-value* Wald value	*p-value*
Camera Visual Surveys			
Season			
- PERMANOVA	1	7.16	**0.0002**
- Negative binomial modeling	1	62.94	**0.001**
Bay			
- PERMANOVA	1	5.44	**0.001**
- Negative binomial modeling	1	40.96	**0.001**
Site			
- PERMANOVA	3	3.07	**0.002**
- Negative binomial modeling	3	87.25	**0.001**
Area			
- PERMANOVA	1	0.70	0.57
- Negative binomial modeling	1	23.09	0.14
Camera Position			
- PERMANOVA	1	9.12	**0.0002**
- Negative binomial modeling	1	30.99	**0.001**
Small Invertebrate Collections			
Season			
- PERMANOVA	1	37.76	**0.0002**
- Negative binomial modeling	1	19.29	**0.001**
Time Period			
- PERMANOVA	3	16.86	**0.0002**
- Negative binomial modeling	3	20.26	**0.001**
Bay			
- PERMANOVA	1	1.65	0.17
- Negative binomial modeling	1	6.43	**0.04**
Site			
- PERMANOVA	3	0.78	0.59
- Negative binomial modeling	3	9.01	**0.02**
Area			
- PERMANOVA	1	0.10	0.98
- Negative binomial modeling	1	1.63	0.99

3.2. Spatial farm effect

All sampling methods - GoPro visual surveys, small invertebrate collectors and eDNA - indicated that there were no significant differences in species richness or in Shannon diversity indices, for both alpha (Tables 5 and 6) and beta (Table 3) diversity, between kelp farms and non-farm reference areas (Fig. 2a, Fig. 3a). Though no spatial differences between kelp farms and non-farm reference areas could be detected, there were some spatial differences detected between sites and vertical camera position (i.e., depth). These spatial differences in species richness and diversity varied temporally and across sampling method.

Significant differences in species richness of large mobile organisms viewed on cameras were found between sites within Casco Bay (Clapboard mean = 3.36 SD ±1.40 (range: 1–7), Chebeague mean = 1.52 SD ±1.21 (range: 0–4)) (Table 5). Significant differences in Shannon diversity indices of large mobile species also exist between sites (Table 5). Specifically, differences were found between Clapboard and Chebeague, Ram and Chebeague, as well as Wood and Chebeague during the summer non-growing season (Clapboard mean = 1.12 SD ±0.42 (range: 0–1.89), Chebeague mean = 0.38 SD ±0.49 (range: 0–1.39), Ram mean = 0.96 SD ±0.49 (range: 0–1.39), Wood mean = 0.73 SD ±0.47 (range: 0–1.39)).

Multivariate analysis showed significant community differences in the types of species between Saco and Casco Bay as well as between sites within the bays (Table 3). There were four organisms significantly driving differences between bays sites: Atlantic rock crabs (*Cancer irroratus*), green crabs (*Carcinus maenas*), Jonah crabs (*Cancer borealis*), and winter flounder (*Pseudopleuronectes americanus*). Casco Bay had higher abundances of Atlantic rock crabs (46 vs. 13 individuals in Saco Bay), green crabs (23 vs. 6 individuals in Saco Bay), and Jonah crabs (37 vs. 17 individuals in Saco Bay). These 3 crabs were also driving differences between sites with higher abundances of these organisms sighted at the co-culture farm off of Clapboard Island; Atlantic rock crabs (Clapboard: 29, Chebeague: 17, Ram: 7, Wood: 6), green crabs (Clapboard: 17, Chebeague: 6, Ram: 4, Wood: 2), and Jonah crabs (Clapboard: 24, Chebeague: 13, Ram: 10, Wood: 7). Whereas winter flounder was seen more in Saco Bay with an abundance of 6, compared to only one (1) winter flounder sighting in Casco Bay. Winter flounder also drove differences between sights with 5 individuals seen at Wood, one (1) seen at Ram, and zero (0) flounder sightings at Clapboard and Chebeague island sites. Similarly, eDNA MiFish NMDS plots show clustering based on site (Fig. 2).

Significant differences in relative abundance of small invertebrates between bays and sites were found (Table 3). However, only one organism, tunicate *Ciona intestinalis*, was found driving these differences. This organism was seen in higher abundances in Casco Bay with 438 individuals collected vs. only one (1) in Saco Bay. This organism also drove differences between sites with higher abundances seen at the Chebeague Island site with 290 individuals collected (Clapboard: 148, Ram: 1, Wood: 0).

Examining a subset of the dataset that consisted of data collected from farm areas only during times that farms were deployed (the winter growing season), allowed us to determine if significant differences in alpha and/or beta diversity were present between cameras position at the benthos and on the grow line (2m from the surface). Significant differences in species richness (p-value = 0.003), but not Shannon diversity indices (p-value = 0.23), were found between camera positions where more species were seen on benthic cameras (benthic mean = 0.74 SD ±0.93 (range: 0–4), surface mean = 0.19 SD ±0.40 (range: 0–1)). Significant differences in beta diversity were also found between camera positions where only one organism, lumpfish (*Cyclopterus lumpus*), was significantly driving 58.9% of differences, with 100% of lumpfish captured on surface cameras. Average dissimilarities using 1- Sørensen index were 0.81 (range 0–1) for camera visual surveys exclusively looking at camera position during the growing season. The eDNA MiFish NMDS plots do not show a relationship between the camera positions (Fig. 2d). Lumpfish were not observed in the eDNA data set despite being represented in the MiFish database and having been observed on cameras.

3.3. Seasonal farm effect

Season is inherently coupled with farming activity and exhibited a strong temporal effect on species richness and biodiversity (Tables 3, 5 and 6, Fig. 3b). Mean species richness for finfish and large crustaceans observed on cameras are 0.65 SD ±0.81 (range: 0–4) for the winter growing season and 2.48 SD ±1.46 (range: 0–7) for the summer non-growing season. Mean Shannon diversity indices for these same species are 0.08 SD ±0.27 (range: 0 = 1.33) in the winter growing season and 0.77 SD ±0.55 (range: 0–1.90) in the summer non-growing season. These seasonal differences were primarily driven by American lobster (*Homarus americanus*), which significantly contributed nearly 50% to the difference in types of species seen between the winter growing and summer non-growing season. Two other organisms, schools of Atlantic menhaden (*Brevoortia tyrannus*), and Atlantic rock crabs (*Cancer irroratus*), significantly contributed 22.8% and 8.4%, respectively, to differences seen between seasons. American lobster and Atlantic menhaden were only seen during summer non-growing seasons, with an abundance of 42 individuals for lobsters and 18 schools of menhaden across both summers. Twelve (12) individual Atlantic rock crabs were seen across both winter growing seasons whereas 47 sightings were made across both summer non-growing seasons.

Similar to the camera visual surveys, season affected small

Table 4
This list of species represents all species collected with small invertebrate devices at any location throughout the study duration (2020–2022). Water samples for eDNA analysis were collected on dates specified in 2021 in Saco Bay, when the invertebrate collectors were retrieved after a ~4 week soak time. Organisms detected by 18S eDNA invertebrate sequencing and PR2 database are shaded grey and organisms observed in collector devices are noted with an "X". There were additional species identified by the 18S eDNA sequencing and PR2 database that are not listed here.

Species	Peak Productivity Apr 07	Peak Biomass May 13	Early Summer Jul 22	Late Summer Aug 27
*Astyris lunata**				X
Calanus sp.	X	X		
Caprella linearis	X	X	X	X
Parvicardium sp.				X
Ciona intestinalus			X	X
Gammarus sp.	X	X	X	
Hiatella arctica			X	X
Idotea baltica		X	X	X
*Lacuna vincta***	X	X	X	X
*Lembos websteri**	X	X	X	X
Mytilus edulis	X	X	X	X
Paracalanus sp.	X	X	X	
Pseudocalanus sp.	X	X	X	
Semibalanus balanoides		X		
Ancinus sp.	X	X	X	

*Genus not included in PR2 database.
***Lacuna* eDNA detected. PR2 database included species *L. pallidula*, and not *L. vincta*, which are highly related at 18S gene.

Table 5
Nested ANOVA results for camera observations of finfish and other large mobile species richness and Shannon diversity indices between seasons (winter growing, summer non-growing), bays (Saco, Casco), sites (Ram, Wood, Clapboard, Chebeague) and areas (farm, non-farm). α = 0.05.

	df	Sum Sq	Mean Sq	F-value	p-value
Species Richness rowhead					
- Season	1	70,927	70,927	107.408	<0.0001
- Bay	2	1332	666	1.008	0.368
- Site	3	18,971	47,443	7.182	<0.0001
- Area	8	3043	380	0.576	0.796
residuals	113	74,619	660		
Shannon Diversity Indices rowhead					
- Season	1	59,512	59,512	109.508	<0.0001
- Bay	2	679	339	0.625	0.537
- Site	4	19,551	4888	8.994	<0.0001
- Area	8	3355	419	0.772	0.628
residuals	113	61,410	543		

Table 6
Nested ANOVA results of collected small invertebrate species richness and Shannon diversity indices between seasons (winter growing, summer non-growing), and time periods (peak seaweed productivity, peak seaweed biomass, early summer, late summer), bays (Saco, Casco), sites (Ram, Wood, Clapboard, Chebeague) and areas (farm, non-farm). α = 0.05.

	df	Sum Sq	Mean Sq	*F-value*	*p-value*
Species Richness					
- Season	1	120.61	120.61	46.903	<**0.0001**
- Time Period	2	25.97	12.99	5.051	**0.022**
- Bay	4	17.77	4.44	1.728	0.199
- Site	8	20.33	2.54	0.988	0.484
- Area	14	31.50	2.25	0.875	0.597
residuals	14	36.00	2.57		
Shannon Diversity Indices					
- Season	1	3928	3928	40.448	<**0.0001**
- Time Period	2	610	305	3.143	0.075
- Bay	4	253	63	0.652	0.635
- Site	8	150	19	0.193	0.987
- Area	14	794	57	0.584	0.837
residuals	14	1230	97		

invertebrate alpha (Table 4) and beta (Table 3) diversity measurements as observed in the small invertebrate collector devices. Results show species richness and Shannon diversity indices approximately 2–3 times higher in the invertebrate collector devices, respectively, during the summer non-growing season (mean richness 7.45 SD ±1.47 (range: 5–11); mean diversity 1.42 SD ±0.31 (range: 0.64 + 1.87)) compared to the winter growing season (mean richness 4.13 SD ±1.98 (range: 1–8); mean diversity 0.53 SD ±0.45 (range: 0–1.61).

Five species – (clam (*Hiatella arctica*), amphipod (*Lembos websteri*), mussel (*Mytilus* edulis), skeleton shrimp (*Caprella linearis*), and copepod (*Pseudocalanus* spp.) – found in the small invertebrate collector devices drove 70.2% of the significant community differences between the winter growing and summer non-growing season, as well as drove 59.5% of the significant differences between time periods within the seasons. Four (4) of these species were found almost exclusively during the summer non-growing season with abundances changing from 4 in the winter to 1619 in the summer for the clam, 14 to 6045 for the amphipod, zero (0) to 283 for the mussel, and 25 to 3536 for the skeleton shrimp. The clam, amphipod, and mussel also drove differences between time periods, peak productivity (PP), peak biomass (PB), early summer (ES), and late summer (LS). These 3 organisms were found in higher abundances during the late summer period; clam (LS: 998, PP: 3, PB: 1, and ES: 621), amphipod (LS: 5,751, PP: 2, PB: 12, ES: 294), mussel (LS: 155, PP: 0, PB: 0, ES: 128). Conversely, copepods (*Psuedocalanus* spp.) were found almost exclusively during the winter non-growing season with abundances of 165 vs. only 9 during the summer non-growing season. Copepod (*Psuedocalanus* spp.) also drove significant

Fig. 2. NMDS plot with 95% confidence interval ellipse visualizing finfish eDNA results using 12S sequencing and MiFish database. There was no difference in organisms found between areas (panel a), between seasons (panel b), or between camera positions (panel d) as shown by the lack of clustering. There is a difference in organisms found between sites, as shown by clustering (panel c).

differences between time periods, being found in higher abundances during the kelp's peak biomass period (PB: 98, PP: 67, ES: 9, LS: 0). The eDNA 18S sequencing invertebrate analysis corroborates with the observational invertebrate collection statistical analysis. NMDS plots using 18S sequencing to detect species show clustering between seasons and time periods within each season (Fig. 3b, d).

4. Discussion

Due to the seasonality of the kelp farming activities, Gulf of Maine kelp farms do not appear to be enhancing habitat for mobile organisms as previously anticipated by other studies. The cold-water conditions needed to cultivate kelp (5–10 °C) (Kim et al., 2015) are opposite to that of many other commercial fisheries species inhabiting the Gulf of Maine, such that many commercially-targeted species migrate offshore during the winter and return in the summer minimizing the temporal overlap in space use of near shore coastal environment. Further contributing to the mismatch in timing and lack of overlap between marine farms and animals is that most of the seaweed farm gear in this region is removed for half of the year to accommodate lobstering, and other commercial fishing activities, making seaweed farms more ephemeral than other types of aquaculture farms. American lobster was the most important organism driving changes between the winter growing and summer non-growing seasons with more lobster seen during the summer non-growing season, when the fishery is active.

Several factors might affect the habitat quality provided by farmed kelp including, local environmental conditions, cultivation gear utilized, farm management practices, species cultivated, and intensity/scale of culture (Theuerkauf et al., 2022). In particular, the structural architecture of suspended kelp near the surface waters, decoupling the kelp biomass from the benthic substrate, could also influence habitat provisioning of kelp farms in the Gulf of Maine. Where kelp biomass is positioned in the water column influences fish recruitment (de Carvalho et al., 2017; Eklöf et al., 2006). For example, red seaweed cultivated on floating rafts showed that the species assemblages were not significantly different between the farm and controls (de Carvalho et al., 2017) whereas farms deployed on the benthos within seagrass beds, found a higher number of species in seaweed farms when compared to controls (Eklöf et al., 2006). Since the historical primary fish assemblage in the Gulf of Maine are benthic and kelp biomass is cultivated near the water's surface, it is not surprising that no significant difference was found between the farms and non-farm reference areas (de Carvalho et al., 2017).

Furthermore, the amount and type of gear in the water may influence species differences. Atlantic rock crabs, green crabs, and Jonah crabs were seen creating significant differences in beta diversity measurements for camera visual surveys between sites. Specifically, all three of these organisms were found in higher amounts around the co-culture farm near Clapboard Island. Species richness was also found significantly higher at Clapboard Island farm when compared to Chebeague Island farm, which was during the summer non-growing season, when the only gear left in the water is the mussel aquaculture rafts. The combination of these results may suggest that the additional gear associated with the co-culture farm may be attracting more and different types of organisms during the summer non-growing season.

When looking in the farm areas, specifically during the winter growing season, both the multivariate statistics and the univariate test of

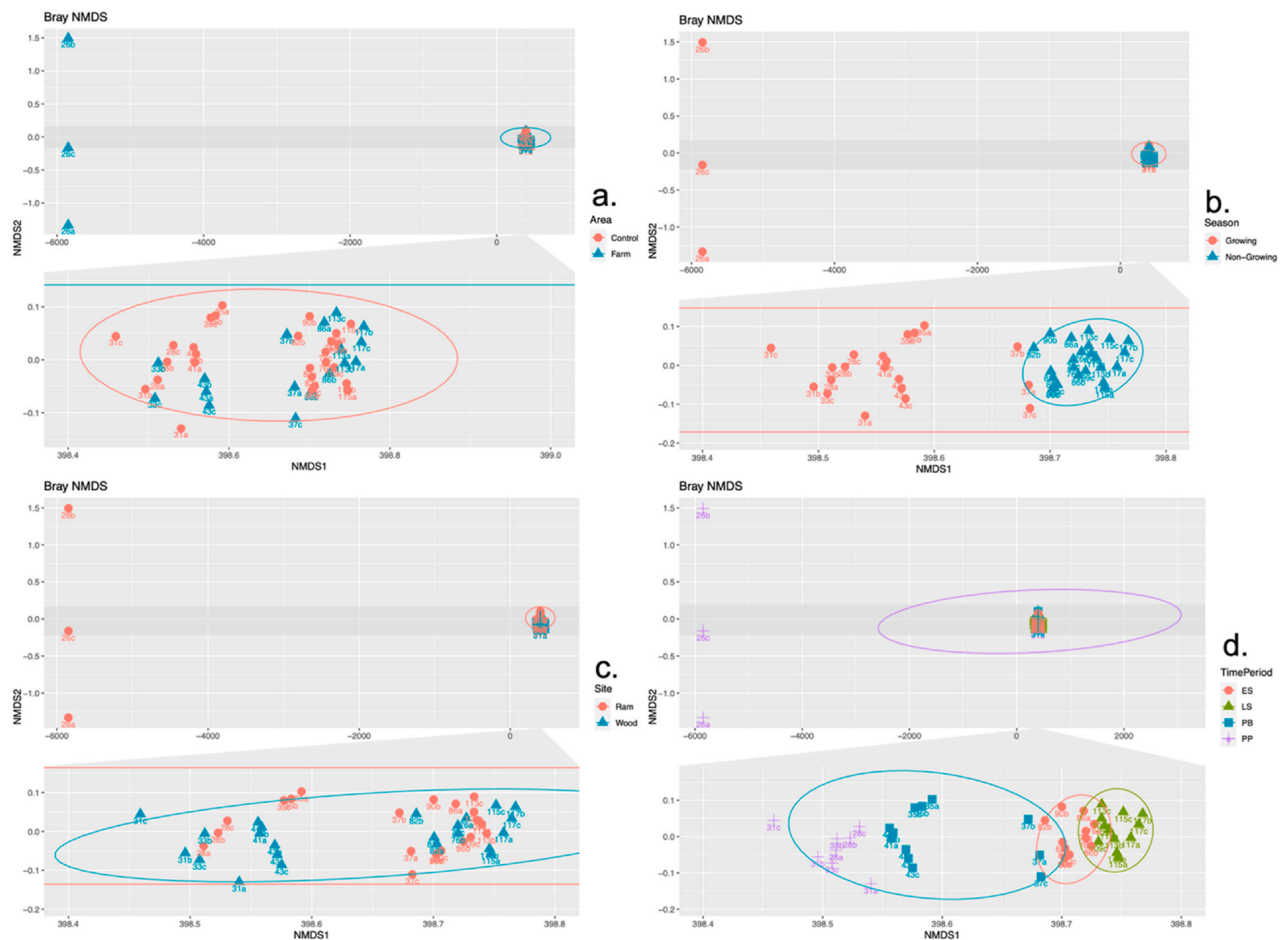

Fig. 3. NMDS plot with 95% confidence interval ellipse visualizing invertebrate eDNA results using 18s sequencing and PR2 database. Spatially, there was no difference in organisms found between areas (panel a) or between sites (panel c), as shown by the lack of clustering. Temporally, there as a difference in organisms found between seasons (panel b) and between time periods (panel d) as shown by clustering (ES early summer; LS late summer; PB peak biomass; PP peak productivity). The outlier samples 26a, 26b, 26c were collected on April 7, 2021 (PP) at the Ram Island farm area in Saco Bay and were identified as entirely *Calanus* copepod. All other samples consisted of a mixture of species.

species richness across camera position indicated a statistically significant difference in species observed between the benthic and surface cameras within a farm. This was not surprising as ground fish were anticipated to be seen and was a major motivation for incorporating benthic cameras into the study design. However, this difference was not driven by abundance of groundfish, but rather by the presence of juvenile lumpfish (*Cyclopterus lumpus*), which were only captured on farm surface cameras sheltering within the kelp biomass. Interestingly, we had a few sightings of mammals on our benthic cameras, namely seals who seemed curious about the deployed camera and field gear.

4.1. Coherence between direct observation and eDNA approaches

eDNA is a relatively new method in marine ecology and has not been readily applied in kelp aquaculture studies. Studies performed in ecosystems where community assemblages are well documented claim that eDNA outperforms traditional survey methods by detecting rare and cryptic species (Gold et al., 2021; Lamy et al., 2021; Liu et al., 2019; Port et al., 2016; Thomsen et al., 2012). As such, this study aimed to determine if eDNA results supported camera visual surveys and small invertebrate findings. With the cameras observing for only a small period of time due to limited battery life and challenging winter conditions for field work, eDNA provides a different way to “look” for organisms with extended residence time (Kelly et al., 2017).

eDNA results show no differences between the farm and non-farm areas, which is the same conclusion from by the camera visual surveys and small invertebrate collections. eDNA also showed differences between seasons for 18S eDNA samples associated with small invertebrates. There was lack of coherence between the visual and eDNA datasets when examining the MiFish 12S eDNA samples for season and camera position. The lack of difference in season and camera position found in the eDNA data, which contrasted the visual camera surveys, was most likely because two large crustaceans, American lobster (*Homarus americanus*) and Atlantic rock crab (*Cancer irroratus*), commonly seen on the cameras were not present in the MiFish database. Important to note is that no lobster or crab species were observed with the 18S sequencing and PR2 database from the surface water collected near the small invertebrate collectors, which is not surprising as large crustaceans are found near the benthos and not in the surface water. Alternatively, the lack of statistical difference in vertical separation between camera positions as examined in the MiFish eDNA data (Fig. 2d) could be an indicator that eDNA methods are not precise over shallow depths. Therefore, the utilization of observational methods, in combination with eDNA methods, proved quite informative in this study.

Despite these few instances where MiFish 12S failed to demonstrate

coherence with observational methods due to the lack of crustaceans in the primer database, we otherwise see strong coherence in the conclusions we draw for the categorical variables we examined. Supporting our primary aim of this study, there was coherence in the conclusions drawn from the observational camera analysis and the 12S MiFish eDNA NMDS analysis in that there was no difference in organisms found at farm and non-farm sites (Fig. 2a), and there were differences in organisms found between the Ram and Wood Island sites (Fig. 2c). There was also demonstration of coherence between the 18S invertebrate sequencing NMDS plots and the small invertebrate collections.

4.2. Conclusion

As natural capital, like wild kelp forests, have been steadily decreasing (Sukhdev et al., 2014), it is important to consider the ecosystem services provided by human-derived systems, such as kelp farms. Farm ecosystems are not a replacement for wild ecosystems, but they do offer some restorative or regenerative properties that can help buffer against climate impact (Forbes et al., 2022; Mizuta et al., 2023). Analyzing the data using multiple methodologies and multiple types of statistical analysis gives us confidence in our conclusions. Seeing a lack of statistical difference in the species richness, biodiversity, presence, and abundance between farm and nonfarm areas suggests that wild organisms are not using Gulf of Maine seaweed aquaculture farms as habitat, which is encouraging news for the aquaculture industry given that most farm gear is removed from the water after harvest. Overall, kelp farms do not appear to be attracting mobile organisms nor driving organisms away as there are few species present during the winter growing season, compared to the summer non-growing season. These findings relay a positive message for seaweed farming in the Gulf of Maine. In other geographies using other methods of farming, the results of this type of study and the implications to industry may be very different. Therefore, it is important to examine supporting ecosystem services, such as habitat provisioning, across a variety of spatial and temporal scales, and across geographies.

Credit author statement

This content has author identifiers in it and should not be included in the peer review process.

Emilly Schutt: Methodology, Data Curation, Writing – Original Draft, Visualization. Rene Francolini: (eDNA) Methodology, Software, Data Curation, Visualization. Nichole Price: Methodology, Formal analysis, Supervision of Schutt. Zachary Olson: (statistical analysis) Formal analysis, Supervision of Schutt. Carrie J. Byron: Conceptualization, Methodology, Resources, Writing – Review & Editing, Visualization, Supervision, Project Administration, Funding acquisition.

Funding

This work was primarily funded by The Nature Conservancy (TNC) and the Builder's Initiative under project name UNE Restorative Kelp Aquaculture. This work was also supported by National Science Foundation award #OIA-1849227 to Maine EPSCoR at the University of Maine through the eDNA Commercialization Seed Grants program. Schutt received additional support from the School of Marine and Environmental Programs and the College of Arts and Sciences at the University of New England (USA). Also funded in part by the World Wildlife Fund with support from the Bezos EarthFund.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Sincere appreciation to The Nature Conservancy, specifically Jocelyn Runnebaum, Heidi Alleway and Robert Jones. Schutt and Byron sincerely thank Adam St. Gelais for advice in field equipment deployment and Doug Rasher for his mentorship in eDNA. Schutt and Byron sincerely thank Hannah Korper and Anna Winkler for their hard work in the lab and in the field collecting samples, preparing gear, and especially for their time sorting, counting, and identifying small invertebrates captured in the collector devices. Thank you to Stuart Ryan for his effort and expertise extracting DNA. Schutt, Byron and Francolini acknowledge Dara Yiu for her contribution curating the MiFish database species list.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2023.106162.

References

Alleway, H.K., Gillies, C.L., Bishop, M.J., Gentry, R.R., Theuerkauf, S.J., Jones, R., 2019. The ecosystem services of marine aquaculture: valuing benefits to people and nature. Bioscience 69 (1), 59–68. https://doi.org/10.1093/BIOSCI/BIY137.

Anderson, M.J., Crist, T.O., Chase, J.M., Vellend, M., Inouye, B.D., Freestone, A.L., Sanders, N.J., Cornell, H.v., Comita, L.S., Davies, K.F., Harrison, S.P., Kraft, N.J.B., Stegen, J.C., Swenson, N.G., 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol. Lett. 14 (1), 19–28. https://doi.org/10.1111/J.1461-0248.2010.01552.X.

Anyango, J.O., Mlewa, C.M., Mwaluma, J., 2017. Abundance, diversity and trophic status of wild fish around seaweed farms in Kibuyuni, South Coast Kenya. 440 ~ International Journal of Fisheries and Aquatic Studies 5 (3), 440–446. www.fisheriesjournal.com.

Augyte, S., Lewis, L., Lin, S., Neefus, C.D., Yarish, C., 2019, 1. Speciation in the Exposed Intertidal Zone: the Case of Saccharina Angustissima Comb. Nov. & Stat. Nov. (Laminariales, Phaeophyceae), vol. 57, pp. 100–112. https://doi.org/10.2216/17-40.1.

Barrett, L.T., Theuerkauf, S.J., Rose, J.M., Alleway, H.K., Bricker, S.B., Parker, M., Petrolia, D.R., Jones, R.C., 2022. Sustainable growth of non-fed aquaculture can generate valuable ecosystem benefits. Ecosyst. Serv. 53, 101396 https://doi.org/10.1016/J.ECOSER.2021.101396.

Beaumont, N.J., Austen, M.C., Mangi, S.C., Townsend, M., 2008. Economic valuation for the conservation of marine biodiversity. Mar. Pollut. Bull. 56 (3), 386–396. https://doi.org/10.1016/J.MARPOLBUL.2007.11.013.

Bekkby, T., Torstensen, R.R.G., Grünfeld, L.A.H., Gundersen, H., Fredriksen, S., Rinde, E., Christie, H., Walday, M., Andersen, G.S., Brkljacic, M.S., Neves, L., Hancke, K., 2023. 'Hanging gardens'—comparing fauna communities in kelp farms and wild kelp forests. Front. Mar. Sci. 10, 1066101 https://doi.org/10.3389/FMARS.2023.1066101/BIBTEX.

Collette, B., Klein-Macphee, G., 2002. In: Bigelow and Schroeder's Fishes of the Gulf of Maine, third ed. Smithsonian Books (Third).

Conover, W.J., Iman, R.L., 1981. Rank transformations as a bridge between parametric and nonparametric statistics. Am. Statistician 35 (3), 124. https://doi.org/10.2307/2683975.

Corrigan, S., Brown, A.R., Ashton, I.G.C., Smale, D.A., Tyler, C.R., 2022. Quantifying habitat provisioning at macroalgal cultivation sites. Rev. Aquacult. 14 (3), 1671–1694. https://doi.org/10.1111/RAQ.12669.

Daily, G., 1997. Introduction: what are ecosystem services? In: Daily, G. (Ed.), Nature's Services: Societal Dependence on Natural Ecosystems. Island Press, pp. 1–10.

de Carvalho, L.L., de Souza, E.G.A., da Mata Jï¿½nior, M.R., Villaï¿½a, R.C., 2017. Assessment of rocky reef fish assemblages close to seaweed farming. Aquacult. Res. 48 (2), 481–493. https://doi.org/10.1111/ARE.12896.

Duarte, C.M., Bruhn, A., Krause-Jensen, D., 2021. A seaweed aquaculture imperative to meet global sustainability targets. Nature Sustainability 2021 5 (3), 185–193. https://doi.org/10.1038/s41893-021-00773-9.

Eklöf, J.S., de La Torre-Castro, M., Nilsson, C., Rönnbäck, P., 2006. How do seaweed farms influence local fishery catches in a seagrass-dominated setting in Chwaka Bay, Zanzibar? Aquat. Living Resour. 19 (2), 137–147. https://doi.org/10.1051/ALR:2006013.

FAO, 2022. The State of World Fisheries and Aquaculture 2022. *The State of World Fisheries and Aquaculture 2022*. https://doi.org/10.4060/CC0461EN.

Flavin, K., Flavin, N., Flahive, B., 2013. Kelp Farming Manual A Guide to the Processes, Techniques, and Equipment for Farming Kelp in New England Waters. https://maineaqua.org/wp-content/uploads/2020/06/OceanApproved_KelpManualLowRez.pdf.

Forbes, H., Shelamoff, V., Visch, W., Layton, C., 2022. Farms and forests: evaluating the biodiversity benefits of kelp aquaculture. J. Appl. Phycol. 1, 1–9. https://doi.org/10.1007/S10811-022-02822-Y/FIGURES/1.

Fujita, R., Augyte, S., Bender, J., Brittingham, P., Buschmann, A.H., Chalfin, M., Collins, J., Davis, K.A., Gallagher, J.B., Gentry, R., Gruby, R.L., Kleisner, K., Moritsch, M., Price, N., Roberson, L., Taylor, J., Yarish, C., 2023. Seaweed blue carbon: ready? Or not? Mar. Pol. 155, 105747 https://doi.org/10.1016/J.MARPOL.2023.105747.

Gentry, R.R., Alleway, H.K., Bishop, M.J., Gillies, C.L., Waters, T., Jones, R., 2020. Exploring the potential for marine aquaculture to contribute to ecosystem services. Rev. Aquacult. 12 (2), 499–512. https://doi.org/10.1111/RAQ.12328.

Gold, Z., Sprague, J., Kushner, D.J., Marin, E.Z., Barber, P.H., 2021. eDNA metabarcoding as a biomonitoring tool for marine protected areas. PLoS One 16 (2), e0238557. https://doi.org/10.1371/JOURNAL.PONE.0238557.

Grebe, G.S., Byron, C.J., Brady, D.C., st Gelais, A.T., Costa-Pierce, B.A., 2021. The effect of distal-end trimming on Saccharina latissima morphology, composition, and productivity. J. World Aquacult. Soc. 52 (5), 1081–1098. https://doi.org/10.1111/JWAS.12814.

Groves, S., 2019. *The Physiological Impacts of Cultivating Mussels on a Kelp Farm: A Solution to Ocean Acidification?* [Undergraduate Thesis]. Mount Holyoke College.

Hasselström, L., Visch, W., Gröndahl, F., Nylund, G.M., Pavia, H., 2018. The impact of seaweed cultivation on ecosystem services - a case study from the west coast of Sweden. Mar. Pollut. Bull. 133, 53–64. https://doi.org/10.1016/J.MARPOLBUL.2018.05.005.

Hehre, E.J., Meeuwig, J.J., 2015. Differential response of fish assemblages to coral reef-based seaweed farming. PLoS One 10 (3), e0118838. https://doi.org/10.1371/JOURNAL.PONE.0118838.

Kelly, R.P., Closek, C.J., O'Donnell, J.L., Kralj, J.E., Shelton, A.O., Samhouri, J.F., 2017. Genetic and manual survey methods yield different and complementary views of an ecosystem. Front. Mar. Sci. 3 (JAN), 283. https://doi.org/10.3389/FMARS.2016.00283/BIBTEX.

Kim, J.K., Kraemer, G.P., Yarish, C., 2015. Use of sugar kelp aquaculture in long island sound and the bronx river estuary for nutrient extraction. Mar. Ecol. Prog. Ser. 531, 155–166. https://doi.org/10.3354/MEPS11331.

Kim, J.K., Stekoll, M., Yarish, C., 2019, 5. Opportunities, Challenges and Future Directions of Open-Water Seaweed Aquaculture in the United States, vol. 58, pp. 446–461. https://doi.org/10.1080/00318884.2019.1625611.

Krause-Jensen, D., Duarte, C.M., 2016. Substantial role of macroalgae in marine carbon sequestration. Nat. Geosci. 9, 737–742. https://doi.org/10.1038/ngeo2790.

Krause-Jensen, D., Lavery, P., Serrano, O., Marba, N., Masque, P., Duarte, C.M., 2018. Sequestration of macroalgal carbon: the elephant in the Blue Carbon room. Biol. Lett. 14 (6), 23955–26900. https://doi.org/10.1098/RSBL.2018.0236.

Lamy, T., Pitz, K.J., Chavez, F.P., Yorke, C.E., Miller, R.J., 2021. Environmental DNA reveals the fine-grained and hierarchical spatial structure of kelp forest fish communities. Scientific Reports 2021 11 (1), 1–13. https://doi.org/10.1038/s41598-021-93859-5.

Liu, Y., Wikfors, G.H., Rose, J.M., McBride, R.S., Milke, L.M., Mercaldo-Allen, R., 2019. Application of environmental DNA metabarcoding to spatiotemporal finfish community assessment in a temperate embayment. Front. Mar. Sci. 6, 674. https://doi.org/10.3389/FMARS.2019.00674/BIBTEX.

McKinley Research Group, L, 2021. Alaska Seaweed Market Assessment | Alaska Fisheries Development Foundation. https://afdf.org/research-library/alaska-seaweed-market-assessment.

MEDMR, 2020. Harvest of Farm-Raised Marine Algae in Maine. https://www.maine.gov/dmr/sites/maine.gov.dmr/files/inline-files/Marine%20Algae%20Table%202015-2020.pdf.

MEDMR, 2022. Aquaculture Map. Department of Marine Resources. https://www.maine.gov/dmr/aquaculture/maine-aquaculture-leases-and-lpas/aquaculture-web-map.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J.Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., Iwasaki, W., 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. R. Soc. Open Sci. 2 (7) https://doi.org/10.1098/RSOS.150088.

Mizuta, D.D., Froehlich, H.E., Wilson, J.R., 2023. The changing role and definitions of aquaculture for environmental purposes. Rev. Aquacult. 15 (1), 130–141. https://doi.org/10.1111/RAQ.12706.

Monuki, K., Barber, P.H., Gold, Z., 2021. eDNA captures depth partitioning in a kelp forest ecosystem. PLoS One 16 (11), e0253104. https://doi.org/10.1371/JOURNAL.PONE.0253104.

Piconi, P., 2020. Edible Seaweed Market Analysis - Island Institute. https://www.islandinstitute.org/edible-seaweed-market-analysis/.

Port, J.A., O'Donnell, J.L., Romero-Maraccini, O.C., Leary, P.R., Litvin, S.Y., Nickols, K.J., Yamahara, K.M., Kelly, R.P., 2016. Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. Mol. Ecol. 25 (2), 527–541. https://doi.org/10.1111/MEC.13481.

Préat, N., de Troch, M., van Leeuwen, S., Taelman, S.E., de Meester, S., Allais, F., Dewulf, J., 2018. Development of potential yield loss indicators to assess the effect of seaweed farming on fish landings. Algal Res. 35, 194–205. https://doi.org/10.1016/J.ALGAL.2018.08.030.

Ricart, A.M., Krause-Jensen, D., Hancke, K., Price, N.N., Masqué, P., Duarte, C.M., 2022. Sinking seaweed in the deep ocean for carbon neutrality is ahead of science and beyond the ethics. Environ. Res. Lett. 17 (8), 081003 https://doi.org/10.1088/1748-9326/AC82FF.

Shannon, C.E., 1948. A mathematical theory of communication. The Bell Syst. Tech. J. 27, 379–423 and 623–656.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.W., Richards, T.A., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. 19 (Suppl. 1), 21–31. https://doi.org/10.1111/J.1365-294X.2009.04480.X.

Stoeckle, M.Y., Adolf, J., Charlop-Powers, Z., Dunton, K.J., Hinks, G., Vanmorter, S.M., 2021. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 78 (1), 293–304. https://doi.org/10.1093/ICESJMS/FSAA225.

Sukhdev, P., Wittmer, H., Miller, D., 2014. The economics of ecosystems and biodiversity (TEEB): challenges and responses. In: Helm, D., Hepburn, C. (Eds.), Nature in the Balance: the Economics of Biodiversity. Oxford University PRess. https://www.teebweb.org/wp-content/uploads/2014/09/TEEB-Challenges-and-Responses.pdf.

Taberlet, P., Coissac, E., Hajibabaei, M., Rieseberg, L.H., 2012. Environmental DNA. Mol. Ecol. 21 (8), 1789–1793. https://doi.org/10.1111/J.1365-294X.2012.05542.X.

Teagle, H., Hawkins, S.J., Moore, P.J., Smale, D.A., 2017. The role of kelp species as biogenic habitat formers in coastal marine ecosystems. J. Exp. Mar. Biol. Ecol. 492, 81–98. https://doi.org/10.1016/J.JEMBE.2017.01.017.

TEEB, 2012. Why Value the Oceans? A Discussion Paper - the Economics of Ecosystems and Biodiversity (TEEB). https://teebweb.org/publications/other/why-value-oceans/.

Theuerkauf, S.J., Barrett, L.T., Alleway, H.K., Costa-Pierce, B.A., st Gelais, A., Jones, R.C., 2022. Habitat value of bivalve shellfish and seaweed aquaculture for fish and invertebrates: pathways, synthesis and next steps. Rev. Aquacult. 14 (1), 54–72. https://doi.org/10.1111/RAQ.12584.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M., Willerslev, E., 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS One 7 (8), e41732. https://doi.org/10.1371/JOURNAL.PONE.0041732.

Thomsen, P.F., Willerslev, E., 2015. Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. 183, 4–18. https://doi.org/10.1016/J.BIOCON.2014.11.019.

Tupper, B., 2023a. BigelowLab/auntie. https://doi.org/10.5281/ZENODO.7767646. *March 2023 Release 1*.

Tupper, B., 2023b. BigelowLab/charlier. https://doi.org/10.5281/ZENODO.7767651. *March 2023 Release 1*.

Tupper, B., Sleith, R.S., 2023a. BigelowLab/dadautils. https://doi.org/10.5281/ZENODO.7767644. *March 2023 Release 1*.

Tupper, B., Sleith, R.S., 2023b. BigelowLab/edna-dada2. https://doi.org/10.5281/ZENODO.7767641. *March 2023 Release 1*.

Turner, C.R., Barnes, M.A., Xu, C.C.Y., Jones, S.E., Jerde, C.L., Lodge, D.M., 2014. Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods Ecol. Evol. 5 (7), 676–684. https://doi.org/10.1111/2041-210X.12206.

Visch, W., Kononets, M., Hall, P.O.J., Nylund, G.M., Pavia, H., 2020. Environmental impact of kelp (Saccharina latissima) aquaculture. Mar. Pollut. Bull. 155, 110962 https://doi.org/10.1016/J.MARPOLBUL.2020.110962.

Walls, A.M., Kennedy, R., Fitzgerald, R.D., Blight, A.J., Johnson, M.P., Edwards, M.D., 2016. Potential novel habitat created by holdfasts from cultivated Laminaria digitata: assessing the macroinvertebrate assemblages. Aquaculture Environment Interactions 8, 157–169. https://doi.org/10.3354/AEI00170.

Weitzman, J., 2019. Applying the ecosystem services concept to aquaculture: a review of approaches, definitions, and uses. Ecosyst. Serv. 35, 194–206. https://doi.org/10.1016/J.ECOSER.2018.12.009.

Westman, W.E., 1977. How much are nature's services worth? Science 197 (4307), 960–964. https://doi.org/10.1126/SCIENCE.197.4307.960/ASSET/46CA756C-8B57-4996-BA24-63A05B035DB7/ASSETS/SCIENCE.197.4307.960.FP.PNG.

White, J.W., Carr, M.H., Caselle, J.E., Palumbi, S.R., Warner, R.R., Menge, B.A., Milligan, K., 2019. SIDEBAR. empirical approaches to measure connectivity. Oceanography 32 (3), 60–61. https://doi.org/10.5670/OCEANOG.2019.311.