



Bacterial community trends associated with sea scallop, *Placopecten magellanicus*, larvae in a hatchery system

Suzanne L. Ishaq^{a,*}, Sarah Hosler^a, Adwoa Dankwa^a, Phoebe Jekielek^b, Damian C. Brady^c, Erin Grey^{d,e}, Hannah Haskell^f, Rachel Lasley-Rasher^f, Kyle Pepperman^g, Jennifer Perry^a, Brian Beal^h, Timothy J. Bowden^a

^a School of Food & Agriculture, University of Maine, Orono, ME 04469, United States

^b Ecology and Environmental Sciences, University of Maine, Orono, ME 04473, United States

^c School of Marine Sciences, Darling Marine Center, University of Maine, Walpole, ME 04573, United States

^d School of Biology and Ecology, University of Maine, Orono, ME 04469, United States

^e Maine Center for Genetics in the Environment, University of Maine, Orono, ME 04469, United States

^f Department of Biological Sciences, University of Southern Maine, Portland, ME 04103, United States

^g Downeast Institute, Beals, ME 04611, United States

^h Division of Environmental & Biological Sciences, University of Maine at Machias, Machias, ME 04654, United States

ARTICLE INFO

Keywords:

Aquaculture microbiome
Host associated microbiome
Bacterial community
Vibrio

ABSTRACT

Atlantic sea scallops, *Placopecten magellanicus*, are the most economically important marine bivalves along the northeastern coast of North America. Wild harvest landings generate hundreds of millions of dollars, and wild-caught adults and juvenile spat are increasingly being cultured in aquaculture facilities and coastal farms. However, the last two weeks of the larval maturation phase in hatcheries are often plagued by large mortality events. Research into other scallop- and aquacultured-species point to bacterial infections or altered functionality of microbial communities which associate with the host. Despite intense filtering and sterilization of seawater, and changing tank water every 48 h, harmful microbes can still persist in biofilms and mortality is still high. There are no previous studies of the bacterial communities associated with the biofilms growing in scallop hatchery tanks, nor studies with wild or hatchery sea scallops. We characterized the bacterial communities in veliger-stage wild or hatchery larvae, and tank biofilms using the 16 S rRNA gene V3-V4 region sequenced on the Illumina MiSeq platform. Hatchery larvae had lower bacterial richness (number of bacteria taxa present) than the wild larvae and tank biofilms, and hatchery larvae had a similar bacterial community (which taxa were present) to both wild larvae and tank biofilms. Bacterial richness and community similarity between tank samples fluctuated over the trial in repeated patterns of rise and fall, which showed some correlation to lunar cycle that may be a proxy for the effects of spring tides and trends in seawater bacteria and phages which are propagated into hatchery tanks. These results along with future work, will inform hatcheries on methods that will increase larval survival in these facilities, for example, implementing additional filtering or avoiding seawater collection during spring tides, to reduce bacterial taxa of concern or promote a more diverse microbial community which would compete against pathogens.

1. Introduction

Scallops are a diverse animal group of marine bivalve mollusks (family *Pectinidae*) with global distribution in coastal waters (Fig. 1).

Atlantic deep-sea scallops, *Placopecten magellanicus*, are found along the eastern coast of the United States and Canada, where they have long been a source of food and economic opportunity (The Centre for Indigenous Peoples' Nutrition and Environment, 2017; Tremblay et al.,

* Corresponding author.

E-mail addresses: sue.ishaq@maine.edu (S.L. Ishaq), shosler@albright.edu (S. Hosler), adwoa.dankwa@maine.edu (A. Dankwa), phoebe.jekielek@maine.edu (P. Jekielek), damian.brady@maine.edu (D.C. Brady), erin.grey@maine.edu (E. Grey), hvhaskell018@gmail.com (H. Haskell), rachel.lasleyrasher@maine.edu (R. Lasley-Rasher), kyle.pepperman@downeastinstitute.org (K. Pepperman), jennifer.perry@maine.edu (J. Perry), bbeal@maine.edu (B. Beal), timothy.bowden@maine.edu (T.J. Bowden).

<https://doi.org/10.1016/j.aqrep.2023.101693>

Received 7 March 2023; Received in revised form 26 July 2023; Accepted 30 July 2023

Available online 3 August 2023

2352-5134/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

2020). Wild populations of scallops are currently at an increased risk of decline because of climate change (Marushka et al., 2019; Rheuban et al., 2018), and on top of natural variation in scallop populations, this makes the industry financially vulnerable (Coleman et al., 2021b; rbouvier Consulting, 2019). Shellfish farms are of significant ecological and economic value, as shellfish farms to remove nitrogen runoff and clean coastal waters (Bayer, 2022a, 2022b), or to create artificial reefs and habitats to improve surrounding biodiversity (Mercaldo-Allen, 2022). However, scallop aquaculture in the Northwest Atlantic, while increasing, is still in its infancy with few farms in commercial operation (Coleman et al., 2021a, 2021b). One of the bottlenecks to production is a lack of hatcheries which is potentially hindered by a lack of understanding the interactions with microorganisms in wild and hatchery settings.

All bivalves have a hinged hard shell (Wang et al., 2017), but sea scallop juveniles and adults (Fig. 1) use developed shells and adductor muscles to exert water pressure to move. Sea scallop adults are sessile but not attached and prefer a sandy or shell hash bottom habitat. Sea scallops are an extremely fecund species: females may release up to 270 million eggs per spawn (Langton et al., 1987), which typically occurs in August - October along the coast of Maine, although scallops may spawn multiple times per year under specific conditions (Bayer et al., 2019; Thompson et al., 2014). Scallops utilize broadcast spawning for reproduction, releasing their eggs and sperm into the water column to passively fertilize. Once fertilized, the larvae (Fig. 1) develop through planktonic, trochophore, and veliger stages for approximately 40 days, during which they are at the mercy of ocean currents and feed on algae in the water column. Veligers will begin to settle to the bottom surface or occasionally recruit together using spun mucus mats and sink to a

surface for attachment (Meredith White, Mook Sea Farms, personal communications), which has been noted in other marine animals (Churchill et al., 2011). By the veliger stage, larvae begin to interact more with biofilms on the bottoms of tanks. "Spat" is the term used to describe the beginning of the juvenile phase (less than 15 mm) (Culliney, 1974; Truesdell, 2014). As spat, mortality rates typically decrease in hatcheries.

Scallops' reproductive potential and industry demand make them a prime target for hatchery- and farm-based production, and this has been successfully achieved in some species, such as bay scallops, as well as to restock wild populations in decline (Tettelbach et al., 2002). Hatcheries collect wild sea scallop adults, or maintain cultured broodstocks, and successfully spawn them in their facilities with the intention of forming a plentiful population to grow to adulthood, spawn, and sell to create a sustainable production cycle while also reducing disruption to the scallops' natural habitat. Unfortunately, in sea scallop hatcheries the last two weeks of the larval maturation phase, the veliger-stage (Fig. 1), is plagued by large mortality events, going from 60 million sea scallop larvae down to several thousand individuals in a span of 48 h (Beal, 2014). Survival of clutches to maturity remains very low, with an industry-standard rate around 1% (Downeast Institute, Maine, unpublished data; Andersen et al., 2011). This drastic winnowing of larvae reduces the availability of cultured sea scallop spat for farmers, forcing sea scallop farms to rely almost exclusively on sea scallop spat collected from wild populations for stock and is seen as a bottleneck for growth of the industry and achieving sustainable harvests.

Hatchery larval die-off is well-demonstrated not to be caused by inadequate diet, lighting, temperature, or atmospheric pressure in aquaculture facilities compared to wild conditions (Culliney, 1974;

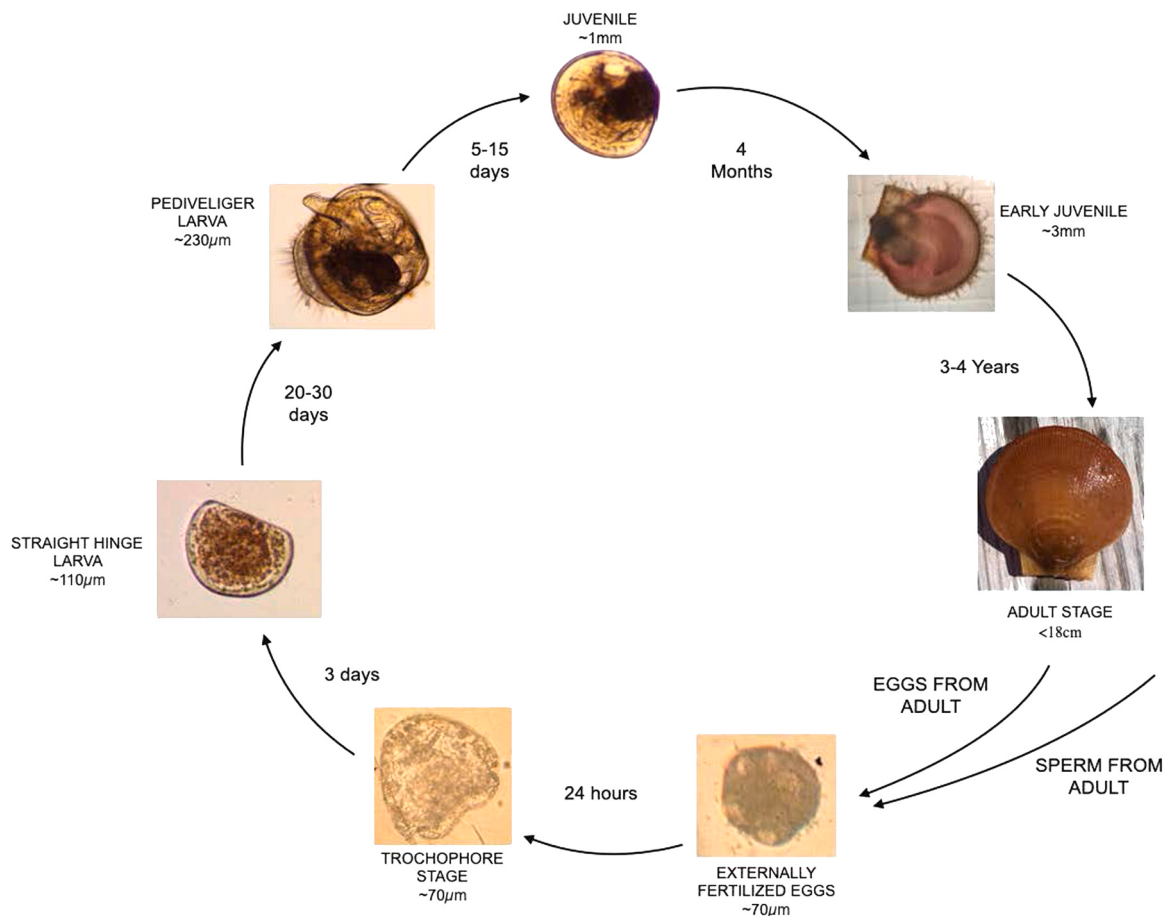


Fig. 1. Life cycle of Atlantic sea scallops. Photo Credits: Downeast Institute, Mook Sea Farms, and Hurricane Island. Image is adapted from a previous version (Packer et al., 1999).

Gouda et al., 2006; Pernet and Tremblay, 2004; Robinson et al., 2016). Scallops are suspension filter feeders and are able to trap plankton and other food particles in mucus; larvae by using particles collected on the velum tissue while swimming, and adults by using particles collected while filtering water through their specialized tissues, or gills. After trapping particles, the scallop moves the food particles towards the mouth using cilia on host tissues. Particles collected into a larger bolus may be passed to the stomach, as they have a segmented organ system, and eventually digested is passed as feces. Alternatively, in adults, a bolus may be rejected and released before digestion as “pseudofeces”. The nature of filter feeding exposes animal tissues to a broad and random variety of microorganisms dispersed in food particles and water. Moreover, the accumulation of undigested feed, dead larvae, and feces in hatchery tanks can support a microbial community which can in turn affect larvae.

To date, there have been no previously published data on the microbial community associated with wild or farmed Atlantic deep-sea scallops, *Placopecten magellanicus*, or on hatchery tank surfaces. In general, there are relatively few studies that explore the microbial community of any scallop species with reported distinctions in the microbial community based on scallop species (Liu et al., 2020), anatomical organ sampled (Ma et al., 2019), and health status (Muñoz et al., 2019; Yu et al., 2019). In other animals, it is known that host species play a crucial role in forming the host-associated microbiome, even among closely related species (Kohl, 2020; Lim and Bordenstein, 2020). Not only are diet and other biological factors selecting for microbiota, but many animal species seed microbial communities into offspring during birth or egg-laying, particularly pectinids (Holbach et al., 2015). However, the environment may play a more important role than host species in acquiring microbes by marine larvae (Boscaro et al., 2022). It is also demonstrated in shrimp facilities that maintaining a healthy microbiome is critical to the success of production (Rajeev et al., 2021).

In this study, we collaborated with scallop hatcheries in Maine to better understand microbial dynamics in hatcheries and scallops. Our objective was to characterize the microbial community present in wild scallop larvae, on tank surfaces, and in hatchery scallop larvae, and assess the contribution of bacteria from tank sources to larval sinks. In addition to describing communities, we hypothesized that bacterial diversity would be lower after tank cleaning, and that bacterial communities in static tanks would be less similar to those in flow-through tanks. We also tested the *a posteriori* hypothesis that bacterial communities were correlated with lunar cycle. The long-term goal of this research is to standardize management practices that support beneficial microbiome assembly to improve animal health, and thereby enhance the success of cultured scallop production.

2. Materials and methods

2.1. Hatchery tank setup

The experimental design and protocols for this project were developed in conjunction with our scallop industry partners to reflect typical management practices and concerns. We collaborated with the Downeast Institute (DEI) on the northern Gulf of Maine coast, (Beals, Maine, US; 44.4806° N, 67.5986° W) to collect cultured larvae from hatcheries and tank biofilm samples (graphical abstract). Following typical production protocols which mimic industry standards, 350 L conical larval development tanks were a static system for the first 10 days of life, at which point larvae were ~150 µm in size (measured along the shortest linear distance in early stages) and large enough to be retained on 75-micron water filters. After 10 days, larvae tanks either stayed in 90 L conical static tanks or were transferred into 90 L conical flow-through tanks with a flow rate of either 1.2 or 0.6 liters per minute (Fig. 2) for the next stages of development, and stocked at starting densities of 5 or

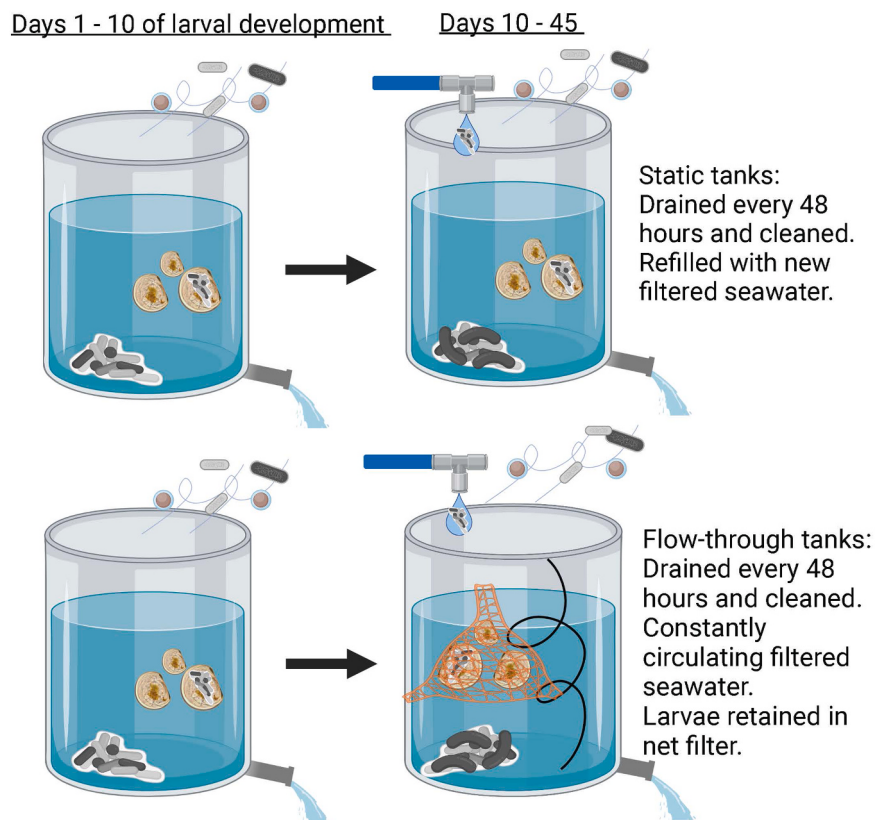


Fig. 2. Scallop larvae development tank design. There were two larval tanks, both were static-water tanks until day 10 of larval development and then one was switched to be a flow-through tank which constantly filters and recirculates. All tanks were drained and cleaned every 48 h. Image created with biorender.

15 larvae per ml. Animals were fed a mixture of *Chaetoceros muelleri*, *Thalassiosira pseudonana*, *Pavlova lutheri*, and *Tisochrysis lutea* cultured microalgae at a density of 30,000 cells per ml of seawater in both static and flow-through tanks. The flow-through tanks filtered circulating water through a 10-micron filter in addition to previous filtering steps, but reported ciliates present in the water which are known to graze on bacteria but act commensally to larvae (Oliva and Sánchez, 2005; Song et al., 2002). Aside from water movement in the tanks, all other environmental and management parameters were the same between the static and flow-through tank treatments.

Biofilm growth was sampled and mitigated every 48 h as follows. Larvae were removed from all tanks with fine filtering (75 µm), water was completely drained out, the tanks were cleaned with soap, water, and a low concentration bleach solution, and larvae were replaced into a fresh tank of filtered seawater. Up to six swabs were collected from distinct areas of the surface of tanks at each of the drawdown events, treating each tank as an ecosystem rather than one experimental unit. Three swabs were collected after the water was drained but prior to cleaning, and three were taken off the tank sides after cleaning and refilling the tanks. Additional swabs were collected from broodstock tanks of adult scallops at the beginning of the trial.

The biofilm swabs from DEI were preserved for bacterial community sequencing. The swab tips were snapped off into 2 ml tubes, immersed in phosphate-buffered solution (PBS, pH = 8.0), and then stored at −20 °C until transported to the University of Maine for DNA extraction and sequencing. The swabs were vortexed for 60 s before DNA extraction to release the bacteria from the swab.

2.2. Larval sampling

To examine the bacterial communities in hatchery D-stage veligers, larvae were collected from tanks at two consecutive draindowns, and placed into four 2-ml tubes (> 20 larvae per tube) and preserved with 70% ethanol at room temperature until transported to the University of Maine. Hatchery eggs were sampled within 48 h of spawn, larvae and eggs within 72 h of spawn. Veligers were 32 days old at the Sept 26 sampling (spawn date Aug 25), and a second cohort were 21 days old at the Oct 16 sampling (spawn Sept 24).

To complement the DEI hatchery larvae samples, we obtained wild veliger samples which had been previously collected in October 2018 from the southern Gulf of Maine in Cape Elizabeth, Maine approximately 5 miles from shore at a station depth of 40 m (43.2925 N, 70.1159 W). We collected larvae using a plankton net with a diameter of 0.5 m and a mesh size of 80 µm. To sample the entire water column, the plankton net was released to approximately one meter above the substrate and then was pulled by hand at a rate of approximately 0.5 m s^{−1}. Once collected, the mixed zooplankton samples were preserved in 70% ethanol and stored at −20 °C. Hinged bivalve larvae were identified according to reference manuals which illustrate mussels but not scallops (Johnson and Allen, 2012; Tremblay et al., 1987), and separated from mixed zooplankton samples under a dissecting microscope at 40x, and moved into ethanol-preserved subsamples using sterile forceps. Based on the time of year, known spawning timing of scallops elsewhere along the coast combined with larval development time, bag position in the water column, and local ecological knowledge from the partner grower (personal communication, Nate Perry), we presumed the veligers to be *P. magellanicus* (Morse et al., 2020). However, to confirm identification, we used a published *P. magellanicus* specific qPCR assay (Bayer et al., 2019). Twelve samples of individual larvae were collected on 10/1/2018, one sample containing multiple larvae on 10/12/2018, and one multiple from 10/26/2018. Samples were stored in 70% ethanol and stored at −20 °C until processing.

2.3. Bacterial community sequencing

Bulk DNA was extracted from veligers, swabs from tank surfaces, or

no-template (water or the ethanol used to preserve wild veligers) control samples (one from each extraction batch) using commercially available kits optimized for water and tissue-based microbial communities (Qiagen Powersoil kit for veligers and Zymo Genomic DNA & Concentrator kit for biofilm swabs), and some aliquots were archived. DNA extract was roughly quantified and purity-checked with a Nanodrop spectrophotometer. Samples underwent DNA amplicon sequencing of the 16 S rRNA gene V3-V4 region, using primers 341 F (Fadrosh et al., 2014) and 806 R (Caporaso et al., 2011) and protocols consistent with The Earth Microbiome Project (The Earth Microbiome Project WWW Document, 2011), and sequenced on an Illumina MiSeq platform using the 2 × 300-nt V3 kit (Molecular Research Labs, Clearwater, TX). The 200 samples and controls were sequenced across two plate batches. Raw sequence data (fastq files and metadata) are publicly available from the NCBI Sequence Read Archive (SRA) under BioProject Accession number PRJNA913436.

Amplicon sequence data was processed to use our previously curated workflows (Supplemental Material), which used the DADA2 pipeline ver. 1.24 (Callahan, 2022) in the R software environment ver. 4.2 (RCoreTeam, 2022). The data from the two sequencing batches were processed for quality control steps separately, and data from both batches were combined prior to rarefaction. We started with 34,123,798 raw paired reads across the two sequencing runs. Trimming parameters were designed based on visual assessment of the aggregated quality scores at each base from all samples (plotQualityProfile in DADA2): the first and last 10 bases were trimmed for batch 1, the last 25 bases were trimmed for batch 2, and sequences were discarded if they had ambiguous bases, more than two expected errors, or matched the phi X genome (used as sequencing positive-control). After filtering, 22,985,705 paired non-unique reads remained.

The DADA algorithm was used to estimate the error rates for the sequencing run, dereplicate the reads, pick sequence variants (SVs) which represent ‘microbial individuals’, and remove chimeric artifacts from the sequence table. Taxonomy was assigned using the Silva taxonomic training data version 138.1 (Pruesse et al., 2007) and reads matching chloroplasts and mitochondria were removed using the dplyr package (Wickham et al., 2015). No-template control samples were used to remove contaminating sequences from the samples by extraction batch (Ishaq, 2017). The sequence table, taxonomy, and metadata were combined using the phyloseq package (McMurdie and Holmes, 2013) to facilitate statistical analysis and visualization, representing 182 samples and 31,451 taxa from 5,107,207 remaining sequences. Due to the large variability in sequences per sample which passed quality assurance parameters (range 42–194,682 sequences/sample), and the knowledge that some sample types would contain much lower microbial diversity than others, the data were rarefied (Cameron et al., 2021; Weiss et al., 2017) to 2988 sequences/sample which was chosen as the cutoff to include all hatchery veliger samples. A total of 164 samples were retained.

Normality was checked using a Shapiro-Wilkes test on alpha diversity metrics generated from rarefied data; observed richness ($W = 0.94167$, $p\text{-value} = 9.934e-05$) and evenness ($W = 0.93813$, $p\text{-value} = 5.849e-05$) were not normally distributed, but Shannon diversity was ($W = 0.98702$, $p\text{-value} = 0.3571$). Linear models were run for comparisons of alpha diversity metrics to compare by sample type. Linear mixed effect models with a sine and cosine function fitted to time (days of trial) was used to study the tank samples as these showed a sine wave-pattern in observed richness (lme4 package (Bates et al., 2015)), in which dirty/clean status and static/flow-through setup were used as fixed and non-interacting factors, and date sampled was a random effect. The emmeans package (Lenth et al., 2019) was used to generate pairwise comparisons of factors in the model and a $t\text{-ratio} > 1.96$ was considered acceptable. Tukey’s HSD was used to adjust for multiple comparisons. A lunar cycle trend was evaluated using lunar phase dates (Yankee Publishing Inc, 2022). Generalized additive models were used to assess trends in alpha diversity using time as a smoother (Pedersen et al.,

2019). Tidal amplitude during the experiment is visualized in Fig. S1. We selected bacterial genera which have been identified as indicators of fecal contamination (*Bifidobacterium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Faecalibacterium*, *Lactobacillus*, *Lactococcus*, *Salmonella*) as a proxy for exploring the hypothesis that lunar high tides increase coastal water contamination (Boehm and Weisberg, 2005; Rochelle-Newall et al., 2015). However, it is worth noting that without culturing to accompany this, we could not determine if these sequences were from viable cells, and if these are strictly human fecal microbiota as some of the genera can be found associated with marine animal hosts.

Jaccard unweighted similarity was used to calculate sample similarity based on community membership (species presence/absence) and non-parametric multidimensional scaling (run 20 stress = 0.2241668, stress type 1 weak ties) and tested with permutational analysis of variance (perMANOVA) by using the vegan package (Oksanen et al., 2020). Beta dispersion, or tightness of community clustering, was tested using beta-dispersion models and Tukey's Honest Significant Difference (HSD) to account for multiple comparisons.

Core taxa were identified using the microbiome package (Lahti and Shetty, 2020), designated as shared across 70% of samples in the subset group comparisons (e.g. wild versus hatchery larvae), and at least 0.001% abundance of the bacterial SV. Random forest feature prediction with permutation was used to identify differentially abundant SVs based on factorial conditions (Archer, 2022). The SourceTracker algorithm (Knights et al., 2011) which had been modified for the R platform was used to identify source:sink effects based on anatomical location. This was used to determine if the cecum could be the source for population sinks in the colon, as a proxy for the model's applicability to the human gut anatomical features and microbial communities. Plots were made using the ggplot2 (Wickham, 2016), ggpvr (Kassambara, 2022), and phyloseq packages. The code for the analysis in the R platform is provided as Supplementary Material.

3. Results

3.1. Wild and hatchery larvae contained distinct richness

The variability in circumstances under which scallop samples were collected prevents a thorough examination of patterns of bacterial richness and diversity; however, bacterial richness (Fig. 3) in wild veligers was higher than in tank systems by an estimated 116 SVs (lm, t. ratio = 2.1, $p = 0.04$), and both sample groups displayed the greatest variability in richness between samples (Figs. 3 and 5). There is a visual

trend implying wild veligers contained more bacterial richness than hatchery-based veligers (Fig. 3), but this was not statistically significant (lm, $p > 0.05$). The evenness of bacterial SV abundance across sample types was not significantly different (lm, $p > 0.05$).

The top 25 most abundant bacterial SVs identified at the genus-level (Fig. 4), as well as all SVs identified at the phylum-level (Fig. S2) demonstrate different bacterial taxa present in wild eggs and larvae compared to hatchery-sourced eggs and larvae. Notably, the genera *Flavobacterium*, *Pseudomonas*, *Psychrobacter*, and *Suttonella* were only found in hatchery-sourced eggs and larvae; and *Pelomonas*, *Polaribacter*, *Porphyromonas*, *Oleispira*, and *Sphingobacterium* were only found in wild caught eggs and larvae.

3.2. Bacterial community richness fluctuated over time

The observed richness in tank surface samples fluctuated over the sea scallop veliger rearing trial (Fig. 5), starting low at the beginning and showing three bell distributions with a 7–12-day period, the vacillation of which generally fits a sine curve distribution. This pattern was muted but still present in samples from late October, which may indicate an underlying sequencing batch effect accounted for during data analysis. The richness in tank biofilms appears to follow the lunar cycle (Sept 7, 2021 - new moon, Sept 13 - first quarter, Sept 20 - full, Sept 28 - last quarter, Oct 6 - new, Oct 12 - first quarter, Oct 20 - full, Oct 28 - last quarter), in which richness is high when the moon is about 50% and richness is low during new and full moon phases. Date was a significant factor (lm or GAM, $p < 0.01$), but we did not find a statistical correlation between richness and lunar phase (Fig. S3; lm or GAM, $p > 0.05$). However, the number of days since the full moon was significantly correlated with bacterial community richness in tanks (Fig. 6, lm or GAM, $p < 0.01$), with a low during the full moon, peaking ~ 21 days after the full moon, and decreasing again just before the next full moon. Using date and days since the full moon together increased the explanatory power of the models, implying both a seasonal and lunar effect.

Days since the last full moon and lunar phase percent were significant factors in explaining the variation in bacterial community similarity among tank surface samples, using a distance-based constrained ordination (Fig. 7, anova $p < 0.01$ for each factor). Samples taken close to full moons clustered together, as did those taken during new moons, and the samples collected at approximately 21 days after a full moon also clustered together.

We used feature prediction algorithms to identify important bacterial SVs based on the number of days since the full moon, to determine if

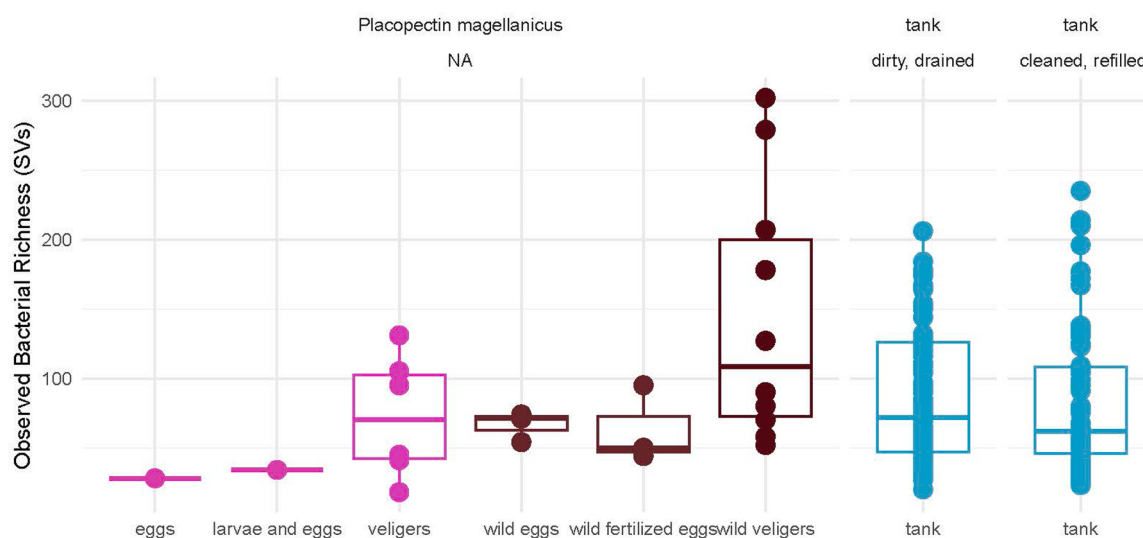


Fig. 3. Bacterial richness of cultured veligers (pink), wild veligers (brown), dirty hatchery tank biofilms (blue, left), and clean hatchery tank biofilms (blue, right). Richness is measured as the number of bacterial sequence variants (SVs).

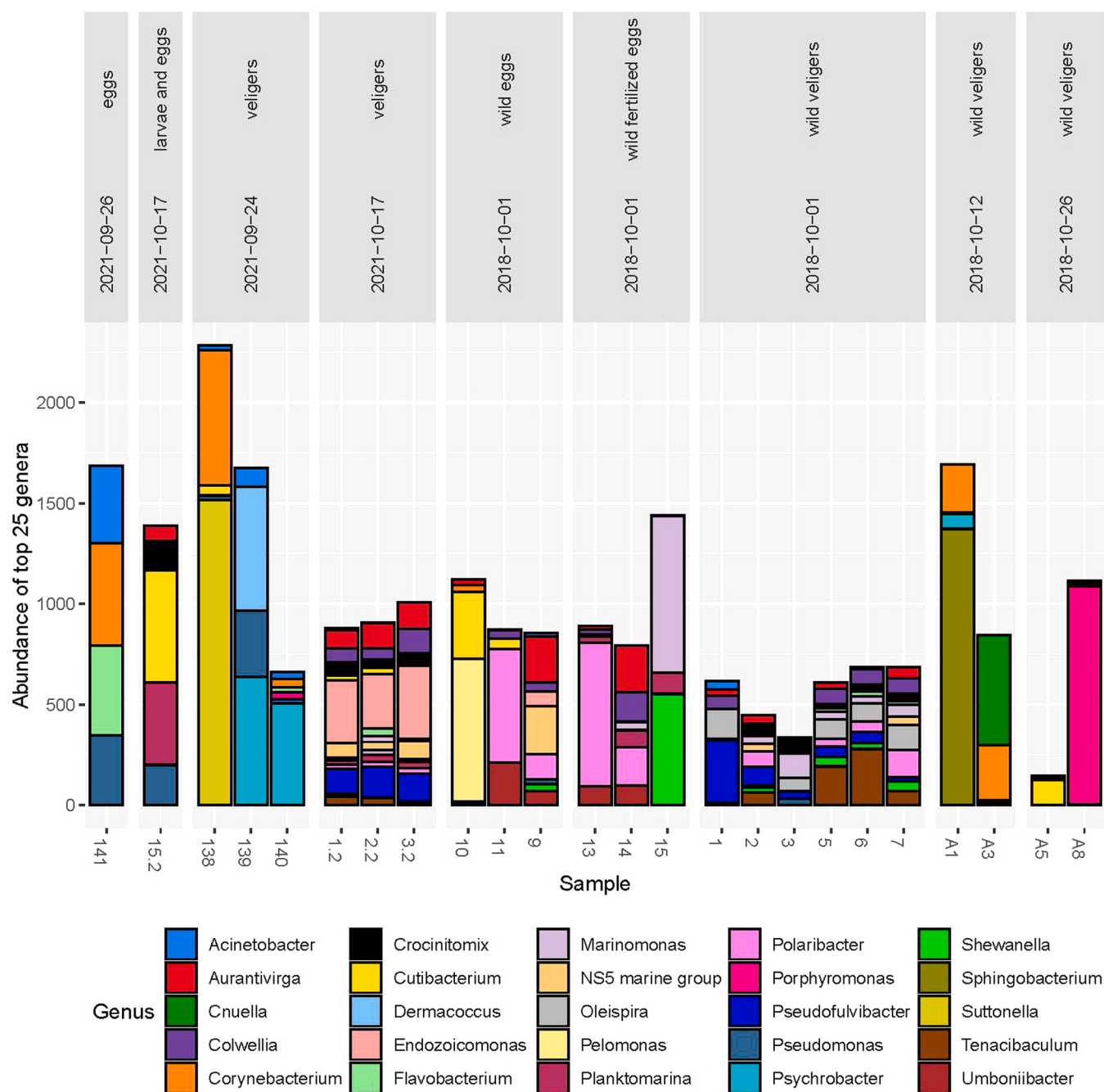


Fig. 4. The top 25 most abundant bacterial sequence variants (SVs) identified at the genus level in wild and hatchery-sourced sea scallop larvae.

certain taxa were time dependent (Fig. 8). *Aureispira* and *Bernardetia litoralis*, common marine bacteria, were relatively high during the full moon, and *Cutibacterium*, *Lawsonella clevelandensis*, and a member of the Comamonadaceae family were present in the week after. Three weeks after the full moon, *Veillonella* and *Pseudomonas* were abundant, and *Acidipropionibacterium* was abundant in the week leading up to the full moon. We selected bacterial genera which have been identified as indicators of fecal contamination, as a proxy in our dataset for exploring the hypothesis that lunar high tides increase coastal water contamination from land-based human waste water (Boehm and Weisberg, 2005; Rochelle-Newall et al., 2015). We found several low-abundance SVs which identified to the *Bifidobacterium*, *Enterococcus*, *Faecalibacterium*, *Lactobacillus*, and *Lactococcus* genera (Fig. S4), but no *Enterobacter*, *Escherichia*, or *Salmonella*. The number of reads from fecal indicator genera was not correlated with tank bacterial richness, number of days

since the last full moon, or lunar phase percent (data not shown). *Lactobacillus* reads were high around the full moon and low or not present at other sampling dates.

3.3. Bacterial communities were not altered by tank water flow or cleanliness

Bacterial richness was not significantly different ($p > 0.05$) between tanks which had been occupied by larvae for 48 h, and which had just been drained, scrubbed clean, and refilled with filtered seawater (Fig. 3, S5), when considering simple comparisons (lm) or complex ones over time (GAM). Richness by tank state was also not different between clean and dirty tanks when data were subset by sequencing batch, and by static/flow-through groups. Cleaning had only a small but significant effect on bacterial community clustering in tanks (Fig. 9) using

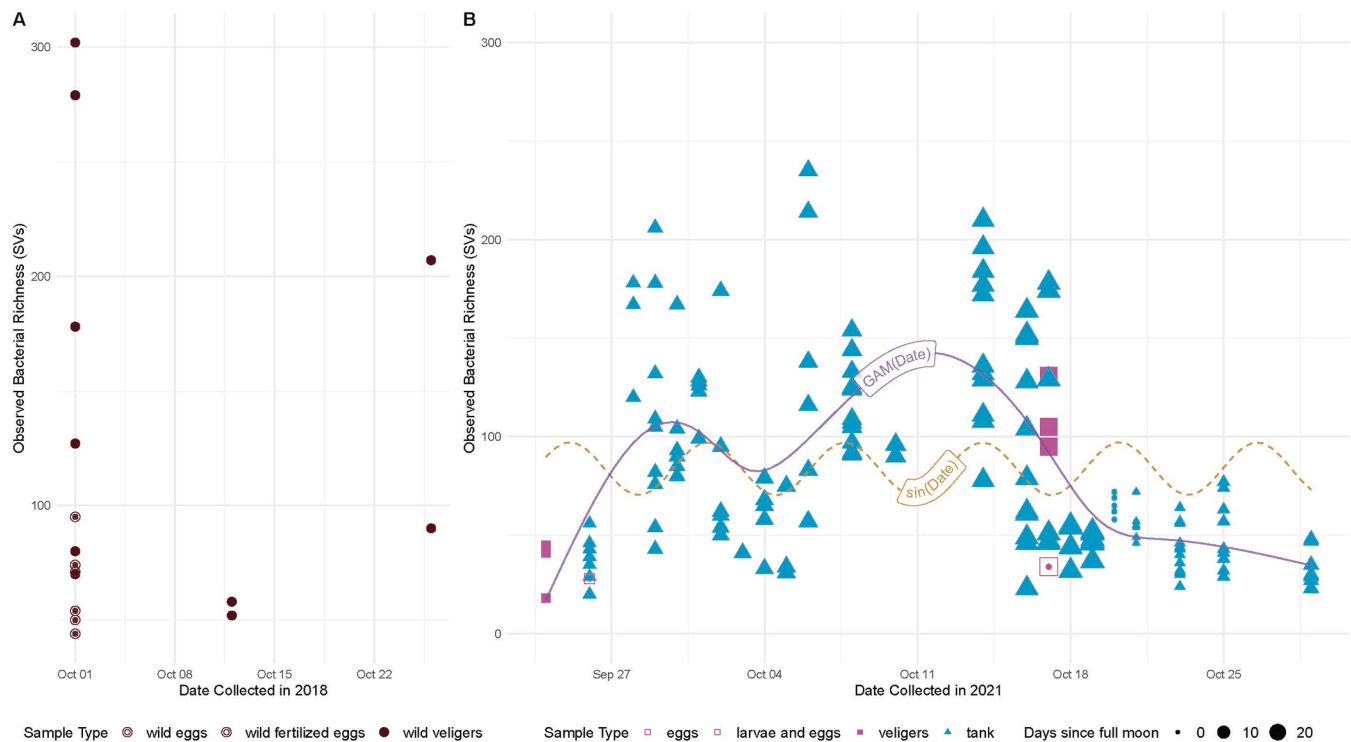


Fig. 5. Observed bacterial richness in (A) wild Atlantic sea scallop veliger-stage larvae or (B) hatchery-based larvae and tank surface biofilms. Color designates sample type, and size of the points in panel B indicates days since the previous full moon. The date of tank surface swabbing is either immediately after filtered seawater has been used to fill the tank (cleaned, refilled) or 48 h after (dirty, drained). In panel B, a generic sine wave by date is visualized in a dotted line, and a general additive model of observed richness by date is visualized in a smooth line. Hatchery eggs were sampled within 48 h of spawn, larvae and eggs within 72 h of spawn. Veligers were 32 days old at the Sept 26 sampling (spawn date Aug 25), and a second cohort were 21 days old at the Oct 16 sampling (spawn Sept 24).

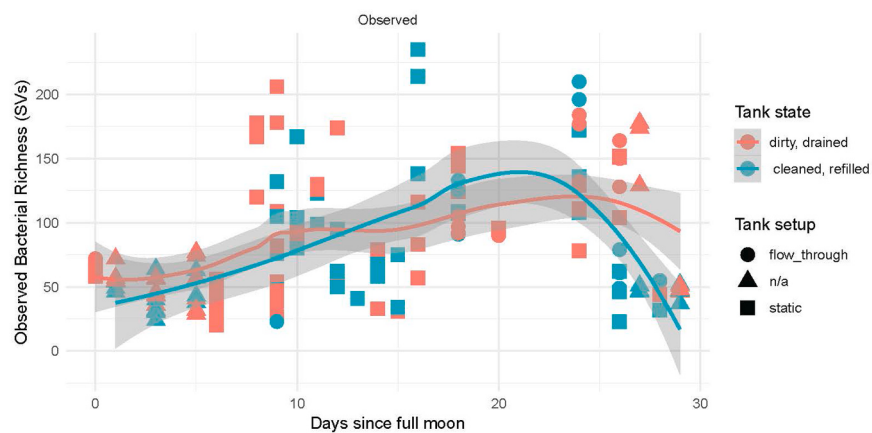


Fig. 6. Bacterial richness in sea scallop hatchery tank biofilm communities compared to the number of days since the last full moon. Richness was measured as the number of bacterial sequence variants (SVs). Tank state describes whether swabbing was either immediately after filtered seawater has been used to fill the tank (cleaned, refilled) or 48 h after (dirty, drained). Tank setup indicates if water was static, constantly filtered and recirculated in a flow-through system, or setup information was not available (n/a). Lines represent the best fit for the data for dirty or cleaned tank samples (method = loess).

unweighted and weighted abundance metrics, and constrained (permanova, $p < 0.05$) and unconstrained (anova, $p < 0.05$) models. This appears to be attributable to differences in the abundance of a few bacterial taxa in specific samples rather than patterns of richness or abundance across the entire group, as random forest feature prediction was only 64% accurate (data not shown).

Static-water-flow compared to continuous-water-flow (flow-through) did not generate different levels of bacterial richness overall (lm, $p > 0.05$), or when comparing only those dates during which both systems were used on larvae of the same age (lm, $p > 0.05$). There was only an equivocal difference when accounting for time as a smoothing feature in the model (GAM, $p = 0.04$). There was a slight difference unconstrained ordination clustering (data not shown) in the presence/absence of bacterial communities between static and flow-through

setups (unweighted Jaccard, permanova, $f = 1.4$, $p = 0.02$), but this was nearly insignificant when accounting for abundance (unweighted Jaccard, permanova, $f = 1.4$, $p = 0.045$) which implies that tanks likely have some rarely abundant bacteria driving the effect in the unweighted comparison.

3.4. Bacterial diversity and richness was different between tanks and veligers

The bacterial communities found in wild veligers were distinct from those found in tanks, using presence/absence (unweighted Jaccard, permanova, $p < 0.001$) or abundance-based metrics of similarity (weighted Bray-Curtis, permanova, $p < 0.001$), and this pattern was observed in both sequencing batches (Fig. 9). Hatchery veliger bacterial

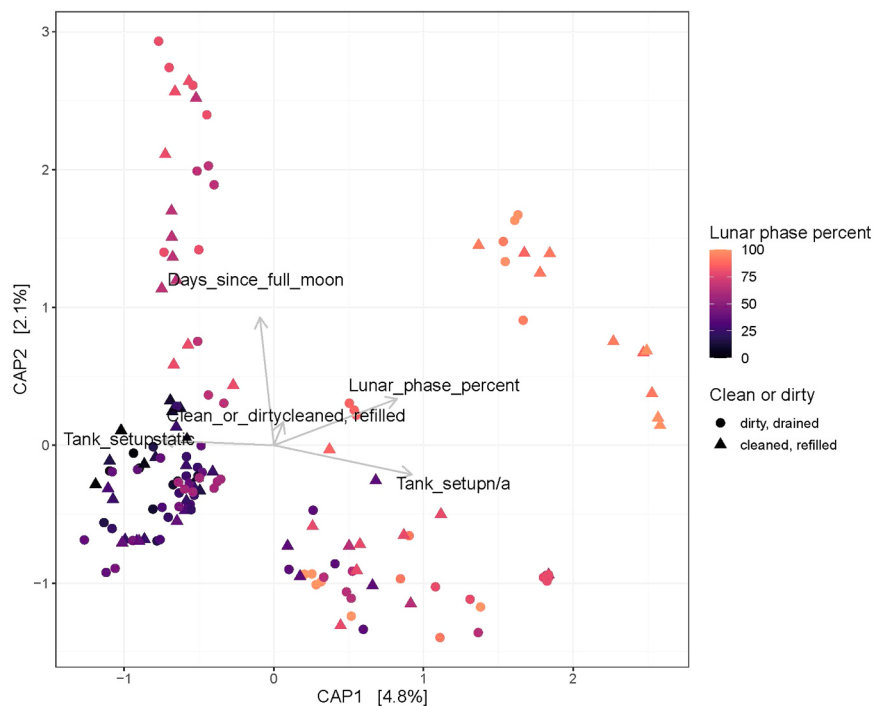


Fig. 7. Constrained ordination of bacterial communities in tank samples. Each point represents the bacterial community from one sample. Similarity between samples was calculated using Distance-based Redundancy Analysis (dbDRA), and significant model factors (anova, $p < 0.01$) are displayed with arrow lengths relative to their importance in the model (f value). The shape of points indicates whether swabbing was either immediately after filtered seawater has been used to fill the tank (cleaned, refilled) or 48 h after (dirty, drained). Tank setup indicates if water was static, constantly filtered and recirculated in a flow-through system, or setup information was not available (n/a).

communities clustered nearby tank samples collected from the same dates. There were 53 bacterial SVs which were determined to be important ($p < 0.05$) to the distinct bacterial community clustering by sample type, with 30% accuracy identifying wild veligers' bacterial communities, and 100% accuracy identifying tanks from biological samples (Fig. S6). Notably, *Fusobacterium* and *Tenacibaculum* were abundant in wild veligers and tanks, and several *Pseudomonas* were abundant in hatchery veligers, tanks, or both.

3.5. Tanks were not an important microbial source for veligers

Despite clustering near to the tank bacterial communities in ordinations, there was no core microbiota (defined as shared by 70% of samples) among all tank and hatchery veligers (data not shown), or at the October timepoint (data not shown). Only a few SVs were shared between veligers and tanks at the September timepoint (Fig. S7), identified as *Corynebacterium*, *Acineobacter*, *Paracoccus*, *Bradyrhizobium*, *Pseudomonas*, *Cloacibacterium*, *Flavobacterium*, and *Vicinamibacteriaceae*. Even fewer bacterial SVs were identified as being sourced from the tank and seeding the microbial community in veligers (Fig. 10). Data were subset to include tank samples taken from the day before or the same day as the two scallop veliger sampling days. The most likely tank-sourced SVs (7 total) were identified as *Fusobacterium*, *Anaerococcus* spp. *Porphyromonas*, and *Pseudomonas*. Scallop veligers were more likely to be the source of bacteria found in tank surfaces (Fig. S8, 19 SVs total), including other strains of *Fusobacterium*, *Porphyromonas*, *Cutibacterium granulosum*, *Desulfomicrobium orale*, and more.

4. Discussion

In this study, we collaborated with a coastal Maine hatchery raising Atlantic sea scallops to identify host-associated bacterial communities in wild and hatchery larvae as well as the bacterial communities of the biofilms in the larvae tanks. To our knowledge, there are no other published studies on *P. magellanicus* microbial communities, although there are some investigating single-microorganism disease dynamics. In other scallop species, bacterial infections have altered the microbial diversity and core bacterial community members (Muñoz et al., 2019;

Yu et al., 2019). Our objective was to identify the microbial community present in wild scallop larvae, on tank surfaces, and in hatchery scallop larvae, and assess the contribution of bacteria from tank sources to larval sinks. The results from 16 S rRNA sequencing revealed trends that hatchery veligers and tanks have lower bacterial richness than wild veligers.

We did not observe many bacterial taxa shared between wild and hatchery-sourced larvae, and no taxa could be considered “core” as none were identified in $> 70\%$ of veliger samples, even when data were subset to compare only wild caught or only hatchery-based samples. The dominant genera in this study were also different from the dominant genera identified in other species of scallops and in other geographic locations (Ma et al., 2019; Yu et al., 2019). Collectively, this suggests a high degree of stochasticity in microbial community acquisition, and a large impact of environmental microbial communities, on marine larvae (Boscaro et al., 2022). The small number of scallop larvae samples available for this study is a limitation, however, these samples do present the first information on bacteria associated with Atlantic sea scallops.

When comparing wild veliger, hatchery veliger, and tank bacterial communities, hatchery veliger bacterial communities appear to be an intermediate between wild veliger and tank bacterial communities. Having similar bacterial taxa to both wild larvae and tank biofilms implies that hatchery-raised veligers are likely acquiring microbiota from broodstock, other larvae, and their environment. The genera *Flavobacterium*, *Pseudomonas*, *Psychrobacter*, and *Suttonella* were only found in hatchery-sourced eggs and larvae, which are commonly found in marine systems and affiliated with marine animals. *Flavobacterium* and *Pseudomonas* are often increased by human activities in marine and other ecosystems (Crone et al., 2020; Nogales et al., 2011), and *Pseudomonas* has been isolated from lesions of diseased Yesso scallops (Liu et al., 2016, 2013; Yu et al., 2019).

Various species of *Vibrio* bacteria have been identified as causative agents in scallop death in hatcheries (Muñoz et al., 2019; Riquelme et al., 1996; Yu et al., 2019). Antibiotics treatment in hatchery systems was explored several decades ago as a means to reduce infections in scallop hatcheries with minimal success, but the inclusion of sediment which would act as a microbial source was also not successful at

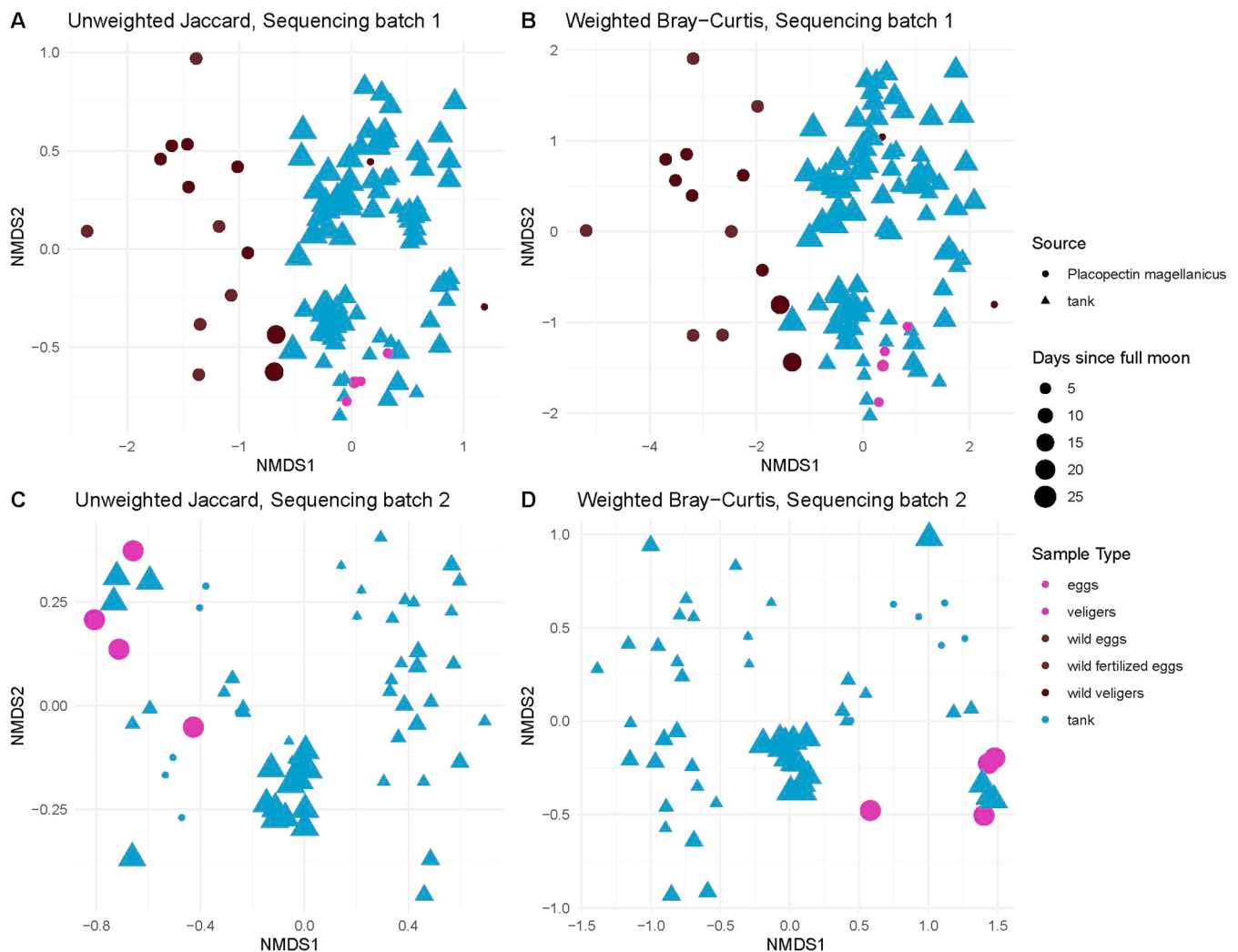


Fig. 8. Relative abundance of bacterial SVs which were significantly differentially abundant by number of days since the previous full moon. The 159 SVs were determined to be differential to samples based on classification by time since the previous full moon, using random forest feature prediction. Only significant features ($p < 0.05$) with more than 50 sequencing reads were visualized.

reducing *Vibrio* contamination (Holbach et al., 2015). In other aquacultured species, the use of bacteria (Richards et al., 2017), algae, and bacteriophages (Richards et al., 2021) against *Vibrio* showed success, highlighting the need for multi-trophic strategies to control infection. We observed relatively few reads identified as *Vibrio* spp. However, insights into *Vibrio* reduction in scallops and other bivalves may help researchers develop ecologically-based management strategies to reduce *Vibrio* or other pathogenic species while providing potentially useful microbial exposure to larvae.

4.1. Microbial connection between wild scallop larvae and their environment

Microbial communities in oceans are volatile over short time periods as water currents (Zorz et al., 2019) and phages targeting microorganisms (Breitbart et al., 2018; Needham et al., 2013) turn over populations in a matter of hours to days. In addition to vertical stratification of communities in the water column (Cui et al., 2019), coastal microbial communities are affected by runoff from human activities (Viau et al., 2011), and even affected by the lunar cycle causing higher tides which can in turn lead to increased connectivity between ocean, estuarine, and land-based sources of nutrients and particulates (Boehm and Weisberg, 2005). Studies of northwestern Atlantic ocean waters show long-term

stability in the community of microorganisms through seasonal trends and continuity in environmental conditions (Needham et al., 2013; Zorz et al., 2019). Yet, as ocean waters continue to warm and acidify, especially in the Gulf of Maine (Pershing et al., 2021), the changing environment may be more suited for different marine microorganisms, and possibly increase the abundance or pathogenic activity of them (Kurpas et al., 2021). Given that we observed time-based and environmental-based effects in microbial communities even in water removed from external conditions, it is likely that viruses and microbiota are surviving the filtration and sterilization of seawater, and that trends in microbial communities in local ocean water will propagate as trends in hatchery tanks where they may affect larval survival.

The microorganisms in ocean waters often seed the microbial communities of marine organisms (Sousa et al., 2021). Wild larvae of various marine species contain a large number of environmentally sourced bacteria (Boscaro et al., 2022), and this may reflect a lack of microbial selection on the part of the host, or the constant influx of environmental microorganisms might overwhelm host-selection trends. Scallops rely on innate mechanisms to maintain homeostasis, including a complex combination of mucus production, phagocytosis, and the production of various cytokines for cell signaling to coordinate host responses (Grayfer et al., 2020; Song et al., 2015). Disease dynamics in wild scallops are still poorly understood, and offer only suggestions of polymicrobial

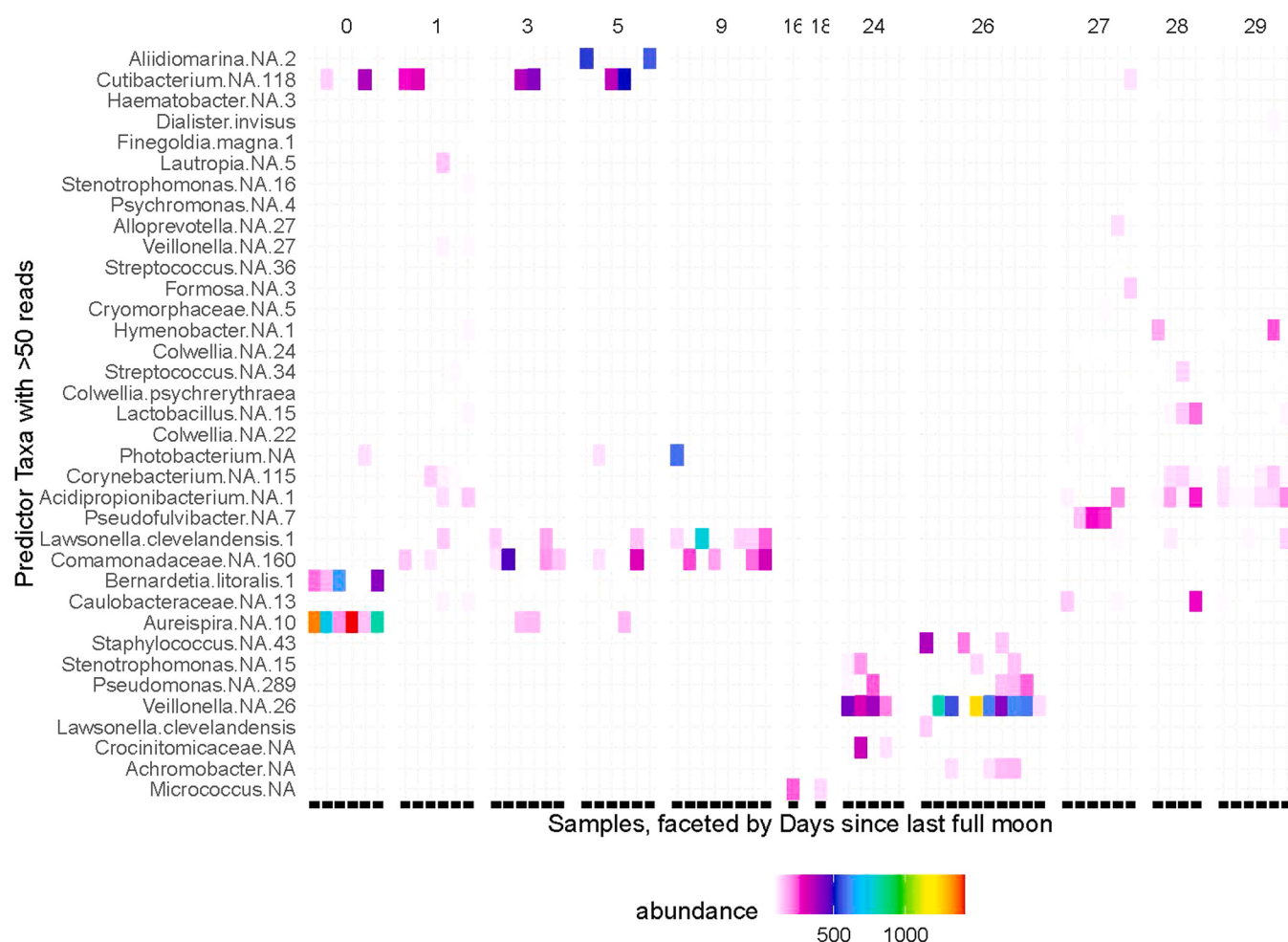


Fig. 9. Non-metric multidimensional scaling (NMDS) plot of bacterial community structure from wild Atlantic sea scallop veliger-stage larvae (brown circles), cultured veliger larvae (pink circles), and tank-associated biofilms (blue triangles). Each point represented the bacterial community from one sample, using different methods of calculating similarity. Data were subset by sequencing batch 1 (A,B) or 2 (C,D). Similarly calculated using unweighted Jaccard similarity to evaluate presence/absence (A, C) or weighted Bray-Curtis to evaluate presence and abundance (B,D).

infections and complex etiological factors of disease, though for many events a causative agent was only putatively assigned (Belvin et al., 2008; Gulka et al., 1983; Gulka and Chang, 1985; Leibovitz et al., 2009; Stokesbury et al., 2007). This hesitancy is partly due to the complexity of tracking infectious disease in wild animal and marine populations, especially without pre-event health monitoring, microbial activity indicators, or the ability to isolate these microorganisms for scallop infection trials. However, if scallop larvae indeed pick up their associated microorganisms from their local environment and exert little to no host selection on that community, that may indicate that the hatchery tank microbial community is a critical source for influencing scallop community acquisition.

4.2. Trends in scallop hatchery tanks

We observed shared bacteria between our hatchery veligers and our tanks. Previous identification of bacterial communities in the water of Yesso scallop (*Patinopecten yessoensis*) hatchery tanks showed community succession in response to scallop larvae stage (Yu et al., 2019). Specifically, *Vibrio*, *Pseudoalteromonas*, and *Sulfitobacter* dominated in the larval trochophore stage, approximately the first 10 days of life, and then *Glaciecola* dominated during the D-veliger stage, days of life ~10–40 (Yu et al., 2019). We found some *Vibrio* and *Pseudoalteromonas* in our tanks, but our tanks had much more *Pseudomonas* across the sampling time.

We also observed a repeating pattern in the rise and fall of bacterial taxa richness in scallop tank surfaces, which correlated to the lunar tidal cycle using statistical analysis on a limited time series, but in the absence of corresponding fresh seawater samples we were unable to confirm this. However, this trend was corroborated by a slight but significant effect of the lunar cycle on beta diversity in our tanks. The hatchery's location on Beal's Island, ME is only 64 km from the mouth of the Bay of Fundy and therefore, has a relatively large tidal range of 3–5 m across a lunar cycle. A Land Ocean Biogeochemical Observatory located in nearby Machias Bay, ME demonstrates that turbidity in this estuary doubles from a neap to a spring tidal cycle and concomitant trends in nitrate and chlorophyll can be observed during this transition (Brady and Maxwell, 2014; Liberti et al., 2022). In short, tidal dynamics in this region are almost certainly a major contributor to particulate and nutrient dynamics which in turn will shape microbial dynamics. The lunar phase has previously been shown to affect California coastal waters with increases in enterococci at the new and full moon stages on a 12-day cycle (Boehm and Weisberg, 2005). Enterococci in land-based waste water can be washed into river and coastal waters during storms in areas without sufficient stormwater and sewage infrastructure (Francy et al., 1993), and can persist in coastal waters which are rich in organic matter and turbid enough to reduce UV damage to microbial cells (Myers and Juhl, 2020; Rochelle-Newall et al., 2015). We found some fecal indicator bacteria in our tank samples, as well as other taxa which were associated with certain times in the lunar cycle. *Aureispira* was high during the full

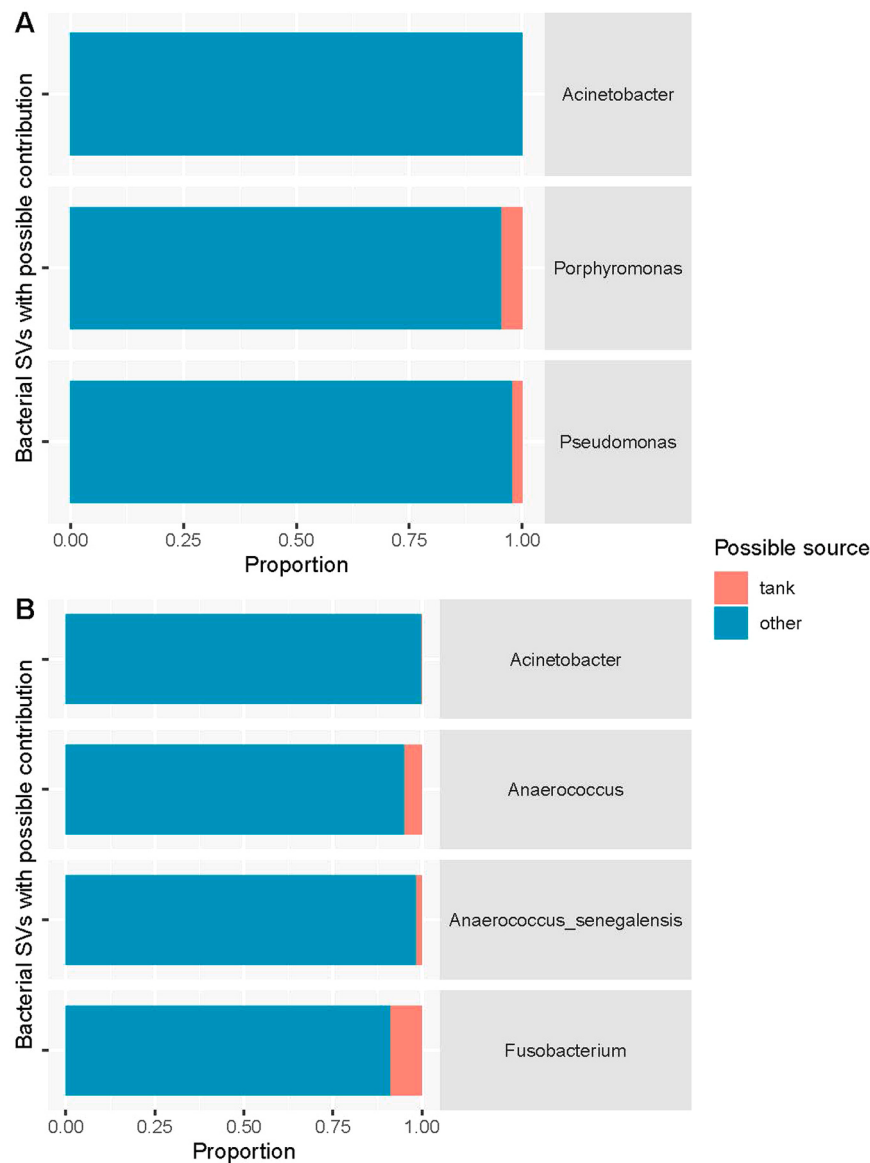


Fig. 10. Bacterial sequence variants from tank surfaces identified as possible sources for bacteria in scallop hatchery veligers in (A) September (B) and October. The SourceTracker algorithm was used to identify bacteria which were significantly ($p < 0.05$) likely to be sourced from tanks and contributed to veliger communities (sinks). Data were subset to include tank samples taken from the day before or the same day as the two scallop veliger sampling days.

moon, and is a predator of *Vibrio* when sufficient calcium is present (Furusawa et al., 2015), which is provided in scallop larval rearing tanks via chemical buffering additives. *Aureispira* and *Bernardetia litoralis* are commonly found in coastal waters. *Cutibacterium* and *Acidipropionibacterium* are commonly found on human skin, and *Lawsonella clevelandensis* has received attention for being pathogenic to humans in rare cases, but has been found in fish previously (Itay et al., 2022). *Veillonella* and *Pseudomonas* are commonly found in all environments, where their behavior and pathogenicity can be context-specific.

Our tank samples were collected 48-hours after fresh ocean water had been filtered and used to refill the tanks, which accounts for the time lag in our richness pattern as compared to the lunar cycle. Additional research is needed to determine if the microbial communities in hatchery veligers would mirror this repeating pattern observed in tanks, and if this creates a biologically meaningful effect for larvae. Many marine animals spawn according to a lunar cycle (Bulla et al., 2017), including scallops in the wild. For example, sperm production is highest around the first quarter but fertilization success is highest in the third quarter (Bayer, 2022a, 2022b; Jekielek, 2022). It is possible that

hatcheries which synchronize their spawning to the lunar cycle would have improved larval survival, if there are as yet unknown cues which the animals respond to or they have developed enough to ward off environmental microorganisms influxing during the lunar high tides.

Ciliates were observed in scallop larval tissues in the flow-through group. Over 150 species of ciliates associate with various scallop species, primarily Trichodinids which have flagella distributed around the cell (peritrichous) (Getchell et al., 2016). Under most conditions, these ciliates are considered to be scallop commensals (Oliva and Sánchez, 2005; Song et al., 2002), and we observed no indication that ciliates were the cause of die-off or large-scale changes to the microbial community. Ciliates often graze on bacteria, and may be feeding inside the developing scallop, and harbor their own microbial community which may be one source for the species of *Pandoraea* bacteria found in tanks (Kostygov et al., 2016). *Pandoraea* are more commonly known for opportunistic infections in cystic fibrosis patients, but it's possible that they can subsist on the mucus chain produced by scallop larvae when they create rafts in preparation to settle.

4.3. Potential benefits to scallop production

The long-term goal of this research is to standardize management practices to support beneficial microbiome assembly to improve animal health, and thereby enhance the success of cultured scallop production and overall aquaculture industry. Globally, scallop meat is a growing commodity (Food and Agriculture Organization of the United Nations, 2018), and the Atlantic sea scallop fishery in the United States is integral to many coastal industries (Agriculture Council of Maine, 2013; Maine Department of Marine Resources, 2020; The Hale Group, 2016). Farmed scallop productions currently rely on collections of wild scallop spat but wild population crashes, habitat quality, harvesting intensity, and, increasingly, ocean acidification and warmer water temperatures (Cooley et al., 2015; Culliney, 1974; Rheuban et al., 2018), all threaten the sustainability and economic viability of this industry (Cooley et al., 2015; Ferraro et al., 2017; Stokesbury et al., 2007). There are considerable year-to-year spatial and temporal fluctuations in natural spat volume which demonstrably results in unpredictable spat supply for the scallop farming sector, and instability for farmers (rbouvier Consulting, 2019; Rheuban et al., 2018; The Hale Group, 2016). If stability in production of cultured juvenile sea scallops could be achieved, total scallop harvest has the potential to grow three-fold in Maine alone over the next 10–15 years (The Hale Group, 2016), and, as an additional benefit, the wild scallop population would have one less stressor. Thus, there is a critical need to decouple this reliance and improve reproducibility of production such that farmed scallop production can become economically viable. If production facilities can take in and store seawater in advance of a lunar high tide, they may be able to avoid the alterations to water chemistry and microbiota in tanks.

CRediT authorship contribution statement

SLI: Conceptualization, Data curation, Funding, Project management, Methodology, Supervision, Resources, Data analysis, Data visualization, Writing, Revisions. **SH:** Data curation, Formal analysis, Investigation, Writing, Revisions. **AD:** Data curation, Formal analysis, investigation, revisions. **DCB:** Conceptualization, Revisions. **EG:** Conceptualization, Funding, Project management, Methodology, Supervision, Resources, Writing, Revisions. **PJ:** data Visualization, Revisions. **KP:** Resources, Supervision, Revisions. **JP:** Conceptualization, Funding, Project management, Methodology, Supervision, Resources, Writing, Revisions. **RL-R:** Resources, Revisions. **BB:** Conceptualization, Funding, Project management, Methodology, Supervision, Resources, Writing, Revisions. **TJB:** Conceptualization, Funding, Project management, Methodology, Resources, Writing, Revisions.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Suzanne L. Ishaq reports financial support was provided by Maine Food and Agriculture Center. Erin Grey reports financial support was provided by National Science Foundation. Editorial Board, Aquaculture Reports: Tim Bowden.

Data Availability

The data are linked in the paper and analysis code is included as supplemental material.

Acknowledgements

The authors would like to thank the staff at the Downeast Institute for supporting the development and implementation of this project, as well as for financially supporting the DNA sequencing; Meredith White of Mook Sea Farm for sharing her expertise and collecting biofilm samples;

the Darling Marine Center for sharing their expertise and collecting biofilm samples; and the Sea Scallop Hatchery Implementation (Hit) Team for their expertise, review of this work, and funding support, who are financially supported by the Atlantic States Marine Fisheries Commission and Michael & Alison Bonney. The authors thank Lilian Nowak for assistance with related lab work to this project, and the Maine Top Scholar Program for related financial support. The authors also thank Nate Perry for helping us collect wild scallop larvae. All authors have read and approved the final manuscript. This project was supported by the USDA National Institute of Food and Agriculture through the Maine Agricultural & Forest Experiment Station, Hatch Project Numbers: ME0-22102 (Ishaq), ME0-22309 (Bowden), and ME0-21915 (Perry); as well through NSF #OIA-1849227 to Maine EPSCoR at the University of Maine (Grey). This project was supported by an Integrated Research and Extension Grant from the Maine Food and Agriculture Center, with funding from the Maine Economic Improvement Fund.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2023.101693.

References

- Agriculture Council of Maine, 2013. Strategic Plan 2013 – 2020 [WWW Document]. Agriculture Council of Maine. URL (<https://maineagcom.org/about-agcom/strategic-plan/>) (Accessed 10.14.19).
- Andersen, S., Christophersen, G., Magnesen, T., 2011. Spat production of the great scallop (*Pecten maximus*): a roller coaster. *Can. J. Zool.* 89, 579–598. <https://doi.org/10.1139/z11-035>.
- Archer, E., 2022. rfrpmute: Estimate Permutation p-Values for Random Forest Importance Metrics.
- Bates, D., Mächler, M., Zurich, E., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.*, Artic. 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Bayer, S., April 27–29 2022a. Defining a model of shellfish nitrogen removal at the oyster farm-scale: an example in Greenwich, Connecticut.
- Bayer, S., April 27–29 2022b. Seven summers of spawning: Methods and lessons learned from spawning sea scallops in mid-coast Maine.
- Bayer, S.R., Countway, P.D., Wahle, R.A., 2019. Developing an eDNA toolkit to quantify broadcast spawning events of the sea scallop *Placopecten magellanicus*: moving beyond fertilization assays. *Mar. Ecol. Prog. Ser.* 621, 127–141. <https://doi.org/10.3354/meps12991>.
- Beal, B.F., 2014. Final Report [NA10NMF4270214]: Enhancing Sea Scallop Stocks in Eastern Maine Through Applied Aquaculture Research and Technology Transfer (No. NA10NMF4270214). Downeast Institute.
- Belvin, S., Tremblay, R., Roussy, M., McGladdery, S.E., 2008. Inoculation experiments to understand mass mortalities in Sea Scallop, *Placopecten magellanicus*. *J. Shellfish Res.* 27, 251–260. [https://doi.org/10.2983/0730-8000\(2008\)27\[251:JETUMM\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2008)27[251:JETUMM]2.0.CO;2).
- Boehm, A.B., Weisberg, S.B., 2005. Tidal forcing of enterococci at marine recreational beaches at fortnightly and semidiurnal frequencies. *Environ. Sci. Technol.* 39, 5575–5583. <https://doi.org/10.1021/es048175m>.
- Boscaro, V., Holt, C.C., Van Steenkiste, N.W.L., Herranz, M., Irwin, N.A.T., Álvarez-Campos, P., Grzelak, K., Holovachov, O., Kerbl, A., Mathur, V., Okamoto, N., Piercey, R.S., Worsaae, K., Leander, B.S., Keeling, P.J., 2022. Microbiomes of microscopic marine invertebrates do not reveal signatures of phyllosymbiosis. *Nat. Microbiol.* 7, 810–819. <https://doi.org/10.1038/s41564-022-01125-9>.
- Brady, D., Maxwell, E., 2014. Maine EPSCoR Land/Ocean Biogeochemical Observatory [WWW Document]. Maine EPSCoR. URL (<http://maine.loboviz.com/>) (Accessed 3.6.23).
- Breitbart, M., Bonnain, C., Malki, K., Sawaya, N.A., 2018. Phage puppet masters of the marine microbial realm. *Nat. Microbiol.* 3, 754–766. <https://doi.org/10.1038/s41564-018-0166-y>.
- Bulla, M., Oudman, T., Bijleveld, A.I., Piersma, T., Kyriacou, C.P., 2017. Marine biorhythms: bridging chronobiology and ecology. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 372 <https://doi.org/10.1098/rstb.2016.0253>.
- Callahan, B., 2022. DADA2: Fast and accurate sample inference from amplicon data with single-nucleotide resolution.
- Cameron, E.S., Schmidt, P.J., Tremblay, B.J.-M., Emelko, M.B., Müller, K.M., 2021. Enhancing diversity analysis by repeatedly rarefying next generation sequencing data describing microbial communities. *Sci. Rep.* 11, 22302. <https://doi.org/10.1038/s41598-021-01636-1>.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* 108, 4516–4522. <https://doi.org/10.1073/pnas.1000080107>.

- Churchill, C.K.C., Ó Foighil, D., Strong, E.E., Gittenberger, A., 2011. Females floated first in bubble-rafting snails. *Curr. Biol.* 21, R802–R803. <https://doi.org/10.1016/j.cub.2011.08.011>.
- Coleman, S., Cleaver, C., Morse, D., Brady, D.C., Kiffney, T., 2021a. The coupled effects of stocking density and temperature on Sea Scallop (*Placopecten magellanicus*) growth in suspended culture. *Aquac. Rep.* 20, 100684. <https://doi.org/10.1016/j.aqrep.2021.100684>.
- Coleman, S., Morse, D., Brayden, W.C., Brady, D.C., 2021b. Developing a bioeconomic framework for scallop culture optimization and product development. *Aquacult. Econ. Manag.* 1–25. <https://doi.org/10.1080/13657305.2021.2000517>.
- Cooley, S.R., Rheuban, J.E., Hart, D.R., Luu, V., Glover, D.M., Hare, J.A., 2015. An integrated assessment model for helping the United States Sea Scallop (*Placopecten magellanicus*) fishery plan ahead for ocean acidification and warming. *PLoS One* 10, 0124145. <https://doi.org/10.1371/journal.pone.0124145>.
- Crone, S., Vives-Flórez, M., Kvich, L., Saunders, A.M., Malone, M., Nicolaisen, M.H., Martínez-García, E., Rojas-Acosta, C., Catalina Gomez-Puerto, M., Calum, H., Whiteley, M., Kolter, R., Bjarnsholt, T., 2020. The environmental occurrence of *Pseudomonas aeruginosa*. *APMIS* 128, 220–231. <https://doi.org/10.1111/apm.13010>.
- Cui, Y., Chun, S.-J., Baek, S.H., Lee, M., Kim, Y., Lee, H.-G., Ko, S.-R., Hwang, S., Ahn, C.-Y., Oh, H.-M., 2019. The water depth-dependent co-occurrence patterns of marine bacteria in shallow and dynamic Southern Coast, Korea. *Sci. Rep.* 9, 9176. <https://doi.org/10.1038/s41598-019-45512-5>.
- Cullinney, J.L., 1974. Larval development of the giant scallop *Placopecten magellanicus* (Gmelin). *Biol. Bull.* 147, 321–332. <https://doi.org/10.2307/1540452>.
- Fadrosch, D.W., Ma, B., Gajer, P., Sengamalai, N., Ott, S., Brotman, R.M., Ravel, J., 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* 2, 6. <https://doi.org/10.1186/2049-2618-2-6>.
- Ferraro, D.M., Trembanis, A.C., Miller, D.C., Rudders, D.B., 2017. Estimates of Sea Scallop (*Placopecten magellanicus*) incidental mortality from photographic multiple before–after–control–impact surveys. *J. Shellfish Res.* 36, 615–626. <https://doi.org/10.2983/035.036.0310>.
- Food and Agriculture Organization of the United Nations, 2018. The State of World Fisheries and Aquaculture 2018: Meeting the sustainable development goals. Food & Agriculture Org., Rome, Italy.
- Francy, D.S., Myers, D.N., Metzker, K.D., 1993. Escherichia Coli and Fecal-Coliform Bacteria as Indicators of Recreational Water Quality. U.S. Department of the Interior, U.S. Geological Survey.
- Furusawa, G., Hartzell, P.L., Navaratnam, V., 2015. Calcium is required for ixotrophy of *Aureispira* sp. CCB-QB1. *Microbiology* 161, 1933–1941. <https://doi.org/10.1099/mic.0.000158>.
- Getchell, R.G., Smolowitz, R.M., McGladdery, S.E., Bower, S.M., 2016. Chapter 10 - Diseases and Parasites of Scallops. In: Shumway, S.E., Parsons, G.J. (Eds.), *Developments in Aquaculture and Fisheries Science*. Elsevier, pp. 425–467. <https://doi.org/10.1016/B978-0-444-62710-0.00010-9>.
- Gouda, R., Kenchington, E., Hatcher, B., Vercaemer, B., 2006. Effects of locally-isolated micro-phytoplankton diets on growth and survival of sea scallop (*Placopecten magellanicus*) larvae. *Aquaculture* 259, 169–180. <https://doi.org/10.1016/j.aquaculture.2006.03.050>.
- Grayfer, L., DeWitte-Ort, S., Edholm, E.-S.I. (Eds.), 2020. *Innate Immunity in Aquatic Vertebrates*. Frontiers Media SA, Lausanne.
- Gulka, G., Chang, P.W., 1985. Pathogenicity and infectivity of a rickettsia-like organism in the sea scallop, *Placopecten magellanicus*. *J. Fish. Dis.* 8, 309–318.
- Gulka, G., Chang, P.W., Marti, K.A., 1983. Prokaryotic infection associated with a mass mortality of the sea scallop, *Placopecten magellanicus*. *J. Fish. Dis.* 6, 355–364. <https://doi.org/10.1111/j.1365-2761.1983.tb00087.x>.
- Holbach, M., Robert, R., Boudry, P., Petton, B., Archambault, P., Tremblay, R., 2015. Scallop larval survival from erythromycin treated broodstock after conditioning without sediment. *Aquaculture* 437, 312–317. <https://doi.org/10.1016/j.aquaculture.2014.12.003>.
- Ishaq, S.L., 2017. Phyloseq dealing with neg controls. Ishaq example.R. Github.
- Itay, P., Shemesh, E., Ofek-Lalzar, M., Davidovich, N., Kroin, Y., Zrihan, S., Stern, N., Diamant, A., Wosnick, N., Meron, D., Tchernov, D., Morick, D., 2022. An insight into gill microbiome of Eastern Mediterranean wild fish by applying next generation sequencing. *Front. Mar. Sci.* 9. <https://doi.org/10.3389/fmars.2022.1008103>.
- Jekielek, P., April 27–29 2022. Evaluating conditioning and spawning cycles in cultured and wild sea scallops, *Placopecten magellanicus*, in Penobscot Bay, ME.
- Johnson, W.S., Allen, D.M., 2012. *Zooplankton of the Atlantic and Gulf Coasts: A Guide to Their Identification and Ecology*. JHU Press, Baltimore, Maryland. <https://doi.org/10.1353/book.19394>.
- Kassambara, A., 2022. ggpubr: “ggplot2” based publication ready plots.
- Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D., Knight, R., Kelley, S.T., 2011. Bayesian community-wide culture-independent microbial source tracking. *Nat. Methods* 8, 761–763. <https://doi.org/10.1038/nmeth.1650>.
- Kohl, K.D., 2020. Ecological and evolutionary mechanisms underlying patterns of phyllosymbiosis in host-associated microbial communities. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 375, 20190251. <https://doi.org/10.1098/rstb.2019.0251>.
- Kostygov, A.Y., Dobáková, E., Grybchuk-Ieremenko, A., Váhala, D., Maslov, D.A., Votýpka, J., Lukeš, J., Yurchenko, V., 2016. Novel trypanosomatid-bacterium association: evolution of endosymbiosis in action. *MBio* 7, e01985. <https://doi.org/10.1128/mBio.01985-15>.
- Kurpas, M., Michalska, M., Zakrzewski, A., Zorena, K., 2021. First report of the presence of *Vibrio vulnificus* in the Gulf of Gdansk. *Int. Marit. Health* 72, 247–251. <https://doi.org/10.5603/IMH.2021.0048>.
- Lahti, L., Shetty, S., 2020. Introduction to the microbiome R package.
- Langton, R.W., Robinson, W.E., Schick, D., 1987. Fecundity and reproductive effort of sea scallops *Placopecten magellanicus* from the Gulf of Maine. *Mar. Ecol. Prog. Ser.* 37, 19–25.
- Leibovitz, L., Schott, E.F., Karney, R.C., 2009. Diseases of wild, captive and cultured scallops. *J. World Mar. Soc.* 15, 267–283. <https://doi.org/10.1111/j.1749-7345.1984.tb00162.x>.
- Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2019. Package “emmeans.”
- Liberti, C.M., Gray, M.W., Mayer, L.M., Testa, J.M., Liu, W., Brady, D.C., 2022. The impact of oyster aquaculture on the estuarine carbonate system. *Elem. (Wash., DC)* 10. <https://doi.org/10.1525/elementa.2020.00057>.
- Lim, S.J., Bordenstein, S.R., 2020. An introduction to phyllosymbiosis. *Proc. Biol. Sci.* 287, 20192900. <https://doi.org/10.1098/rspb.2019.2900>.
- Liu, H., Tan, K.S., Zhang, X., Zhang, H., Cheng, D., Ting, Y., Li, S., Ma, H., Zheng, H., 2020. Comparison of gut microbiota between golden and brown noble Scallop *Chlamys nobilis* and its association with carotenoids. *Front. Microbiol.* 11, 36. <https://doi.org/10.3389/fmicb.2020.00036>.
- Liu, R., Qiu, L., Yu, Z., Zi, J., Yue, F., Wang, L., Zhang, H., Teng, W., Liu, X., Song, L., 2013. Identification and characterisation of pathogenic *Vibrio splendidus* from Yesso scallop (*Patinopecten yessoensis*) cultured in a low temperature environment. *J. Invertebr. Pathol.* 114, 144–150. <https://doi.org/10.1016/j.jip.2013.07.005>.
- Liu, R., Qiu, L., Zhao, X., Zhang, H., Wang, L., Hou, Z., Gao, D., Song, L., 2016. Variation analysis of pathogenic *Vibrio* spp. and *Pseudomonas* spp. in Changhai mollusc farming waters using real-time PCR assay during 2011–2014. *Mar. Biol. Res.* 12, 146–157. <https://doi.org/10.1080/17451000.2015.1099679>.
- Ma, Y., Li, M., Sun, J., Hao, Z., Liang, J., Zhao, X., 2019. Characterization of bacterial community associated with four organs of the Yesso Scallop (*Patinopecten yessoensis*) by High-Throughput Sequencing. *J. Ocean Univ. China* 18, 493–500. <https://doi.org/10.1007/s11802-019-3791-z>.
- Maine Department of Marine Resources, 2020. Commercial Fishing Historical Landings Data [WWW Document]. Maine Department of Marine Resources. URL (<https://www.maine.gov/dmr/commercial-fishing/landings/historical-data.html>) (Accessed 4.7.20).
- Marushka, L., Kenny, T.-A., Batal, M., Cheung, W.W.L., Fediuk, K., Golden, C.D., Salomon, A.K., Sadik, T., Weatherdon, L.V., Chan, H.M., 2019. Potential impacts of climate-related decline of seafood harvest on nutritional status of coastal First Nations in British Columbia, Canada. *PLoS One* 14, e0211473. <https://doi.org/10.1371/journal.pone.0211473>.
- McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8, e61217. <https://doi.org/10.1371/journal.pone.0061217>.
- Mercado-Allen, R., April 27–29 2022. Shelf and bag oyster aquaculture cages provide habitat for fish similar to natural boulder reefs.
- Morse, D.L., Cowperthwaite, H.S., Perry, N., Britsch, M., 2020. *Methods and materials for aquaculture production of sea scallops (Placopecten magellanicus)*. Maine Sea Grant.
- Muñoz, K., Flores-Herrera, P., Gonçalves, A.T., Rojas, C., Yañez, C., Mercado, L., Brokordt, K., Schmitt, P., 2019. The immune response of the scallop *Argopecten purpuratus* is associated with changes in the host microbiota structure and diversity. *Fish. Shellfish Immunol.* 91, 241–250. <https://doi.org/10.1016/j.fsi.2019.05.028>.
- Myers, E.M., Juhl, A.R., 2020. Particle association of *Enterococcus* sp. increases growth rates and simulated persistence in water columns of varying light attenuation and turbulent diffusivity. *Water Res.* 186, 116140. <https://doi.org/10.1016/j.watres.2020.116140>.
- Needham, D.M., Chow, C.-E.T., Cram, J.A., Sachdeva, R., Parada, A., Fuhrman, J.A., 2013. Short-term observations of marine bacterial and viral communities: patterns, connections and resilience. *ISME J.* 7, 1274–1285. <https://doi.org/10.1038/ismej.2013.19>.
- Nogales, B., Lanfranconi, M.P., Piña-Villalonga, J.M., Bosch, R., 2011. Anthropogenic perturbations in marine microbial communities. *FEMS Microbiol. Rev.* 35, 275–298. <https://doi.org/10.1111/j.1574-6976.2010.00248.x>.
- Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. *Vegan: Community Ecology Package*.
- Oliva, M.E., Sánchez, M.F., 2005. Metazoan parasites and commensals of the northern Chilean scallop *Argopecten purpuratus* (Lamarck, 1819) as tools for stock identification. *Fish. Res.* 71, 71–77. <https://doi.org/10.1016/j.fishres.2004.07.009>.
- Packer, D.B., Cargnelli, L.M., Griesbach, S.J., Shumway, S.E., 1999. *Essential Fish Habitat Source Document: Sea Scallop, Placopecten magellanicus, Life History and Habitat Characteristics* (No. NMFS-NE-134). NOAA.
- Pedersen, E.J., Miller, D.L., Simpson, G.L., Ross, N., 2019. Hierarchical generalized additive models: an introduction with mgcv. *PeerJ* 7, e6876. <https://doi.org/10.7717/peerj.6876>.
- Pernet, F., Tremblay, R., 2004. Effect of varying levels of dietary essential fatty acid during early ontogeny of the sea scallop *Placopecten magellanicus*. *J. Exp. Mar. Biol. Ecol.* 310, 73–86. <https://doi.org/10.1016/j.jembe.2004.04.001>.
- Pershing, A.J., Alexander, M.A., Brady, D.C., Brickman, D., Curchitser, E.N., Diamond, A.W., McClenahan, L., Mills, K.E., Nichols, O.C., Pendleton, D.E., Record, N.R., Scott, J.D., Staudinger, M.D., Wang, Y., 2021. Climate impacts on the Gulf of Maine ecosystem. *Elementa* 9. <https://doi.org/10.1525/elementa.2020.00076>.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 35, 7188–7196. <https://doi.org/10.1093/nar/gkm864>.
- Rajeev, R., Adithya, K.K., Kiran, G.S., Selvin, J., 2021. Healthy microbiome: a key to successful and sustainable shrimp aquaculture. *Rev. Aquac.* 13, 238–258. <https://doi.org/10.1111/raq.12471>.

- rbouvier Consulting, 2019. Market Analysis of Maine Farm-Raised Sea Scallops. Coastal Enterprises, Inc.
- RCoreTeam, 2022. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rheuban, J.E., Doney, S.C., Cooley, S.R., Hart, D.R., 2018. Projected impacts of future climate change, ocean acidification, and management on the US Atlantic sea scallop (*Placopecten magellanicus*) fishery. *PLoS One* 13, e0203536. <https://doi.org/10.1371/journal.pone.0203536>.
- Richards, G.P., Watson, M.A., Needleman, D.S., Uknalis, J., Boyd, E.F., Fay, J.P., 2017. Mechanisms for *Pseudoalteromonas piscicida*-Induced Killing of Vibrios and Other Bacterial Pathogens. *Appl. Environ. Microbiol.* 83. <https://doi.org/10.1128/AEM.00175-17>.
- Richards, G.P., Watson, M.A., Madison, D., Soffer, N., Needleman, D.S., Soroka, D.S., Uknalis, J., Baranzoni, G.M., Church, K.M., Polson, S.W., Elston, R., Langdon, C., Sulakvelidze, A., 2021. Bacteriophages against *Vibrio coralliilyticus* and *Vibrio tubiashii*: isolation, characterization, and remediation of larval oyster mortalities. *Appl. Environ. Microbiol.* 87, e00008–e00021. <https://doi.org/10.1128/AEM.00008-21>.
- Riquelme, C., Toranzo, A.E., Barja, J.L., Vergara, N., Araya, R., 1996. Association of *Aeromonas hydrophila* and *Vibrio alginolyticus* with Larval Mortalities of Scallop (*Argopecten purpuratus*). *J. Invertebr. Pathol.* 67, 213–218. <https://doi.org/10.1006/jjipa.1996.0035>.
- Robinson, S.M.C., Parsons, G.J., Davidson, L.-A., Shumway, S.E., Blake, N.J., 2016. Chapter 18 - Scallop Aquaculture and Fisheries in Eastern North America. In: Shumway, S.E., Parsons, G.J. (Eds.), *Developments in Aquaculture and Fisheries Science*. Elsevier, pp. 737–779. <https://doi.org/10.1016/B978-0-444-62710-0.00017-1>.
- Rochelle-Newall, E., Nguyen, T.M.H., Le, T.P.Q., Sengtaheuanghoung, O., Ribolzi, O., 2015. A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. *Front. Microbiol.* 6, 308. <https://doi.org/10.3389/fmicb.2015.00308>.
- Song, L., Wang, L., Zhang, H., Wang, M., 2015. The immune system and its modulation mechanism in scallop. *Fish. Shellfish Immunol.* 46, 65–78. <https://doi.org/10.1016/j.fsi.2015.03.013>.
- Song, W., Al-Rasheid, K.A.S., Hu, X., Arabia, S., 2002. Notes on the poorly-known Marine Peritrichous Ciliate, *Zoothamnium plumula* Kahl, 1933 (Protozoa: Ciliophora), an ectocommensal organism from cultured scallops in Qingdao, China. *Acta Protozool.* 41, 1.
- Sousa, R., Vasconcelos, J., Vera-Escalona, I., Delgado, J., Freitas, M., González, J.A., Riera, R., 2021. Major ocean currents may shape the microbiome of the topshell *Phorcus sauciatius* in the NE Atlantic Ocean. *Sci. Rep.* 11, 12480. <https://doi.org/10.1038/s41598-021-91448-0>.
- Stokesbury, K.D.E., Harris, B.P., Marino II, M.C., Nogueira, J.I., 2007. Sea scallop mass mortality in a Marine Protected Area. *Mar. Ecol. Prog. Ser.* 349, 151–158. <https://doi.org/10.3354/meps07113>.
- Tettelbach, S.T., Smith, C.F., Wenzel, P., Decort, E., 2002. Reproduction of hatchery-reared and transplanted wild bay scallops, *Argopecten irradians irradians*, relative to natural populations. *Aquac. Int* 10, 279–296. <https://doi.org/10.1023/A:1022429500337>.
- The Centre for Indigenous Peoples' Nutrition and Environment, 2017. *Traditional Animal Foods of Indigenous Peoples of Northern North America*. McGill University Press, Montreal.
- The Earth Microbiome Project [WWW Document], 2011. The Earth Microbiome Project. URL (<http://www.earthmicrobiome.org>).
- The Hale Group, 2016. *Maine Farmed Shellfish Market Analysis*. The Gulf of Maine Research Institute.
- Thompson, K.J., Inglis, S.D., Stokesbury, K.D.E., 2014. Identifying spawning events of the Sea Scallop *Placopecten magellanicus* on Georges Bank. *J. Shellfish Res.* 33, 77–87. <https://doi.org/10.2983/035.033.0110>.
- Tremblay, M.J., Meade, L.D., Hurley, G.V., 1987. Identification of Planktonic Sea Scallop Larvae (*Placopecten magellanicus*) (Gmelin). *Can. J. Fish. Aquat. Sci.* 44, 1. <https://doi.org/10.1139/f87-162>.
- Tremblay, R., Landry-Cuerrier, M., Humphries, M.M., 2020. Culture and the social-ecology of local food use by Indigenous. *Ecol. Soc.* 25, 8.
- Truesdell, S.B., 2014. Distribution, population dynamics and stock assessment for the Atlantic sea scallop (*Placopecten magellanicus*) in the Northeast US.
- Viau, E.J., Lee, D., Boehm, A.B., 2011. Swimmer risk of gastrointestinal illness from exposure to tropical coastal waters impacted by terrestrial dry-weather runoff. *Environ. Sci. Technol.* 45, 7158–7165. <https://doi.org/10.1021/es200984b>.
- Wang, S., Zhang, J., Jiao, W., Li, J., Xun, X., Sun, Y., Guo, X., Huan, P., Dong, B., Zhang, L., Hu, X., Sun, X., Wang, J., Zhao, C., Wang, Y., Wang, D., Huang, X., Wang, R., Lv, J., Li, Y., Zhang, Z., Liu, B., Lu, W., Hui, Y., Liang, J., Zhou, Z., Hou, R., Li, X., Liu, Y., Li, H., Ning, X., Lin, Y., Zhao, L., Xing, Q., Dou, J., Li, Y., Mao, J., Guo, H., Dou, H., Li, T., Mu, C., Jiang, W., Fu, Q., Fu, X., Miao, Y., Liu, J., Yu, Q., Li, R., Liao, H., Li, X., Kong, Y., Jiang, Z., Chourrout, D., Li, R., Bao, Z., 2017. Scallop genome provides insights into evolution of bilaterian karyotype and development. *Nat. Ecol. Evol.* 1, 120. <https://doi.org/10.1038/s41559-017-0120>.
- Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J.R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E.R., Knight, R., 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* 5, 27. <https://doi.org/10.1186/s40168-017-0237-y>.
- Wickham, H., 2016. *ggplot2: Elegant graphics for Data Analysis*. Springer Publishing Company, New York.
- Wickham, H., Francois, R., Henry, L., Müller, K., 2015. dplyr: A Grammar of Data Manipulation. R package version 0.4.3. R Found. Stat. Comput, Vienna. (<https://CRAN.R-project.org/package=dplyr>).
- Yankee Publishing Inc, 2022. Moon Phases and Lunar Calendar for Portland, ME [WWW Document]. Almanac.com. URL (<https://www.almanac.com/astronomy/moon/calendar/ME/Portland/2021-10>) (Accessed 8.26.22).
- Yu, Z., Liu, C., Fu, Q., Lu, G., Han, S., Wang, L., Song, L., 2019. The differences of bacterial communities in the tissues between healthy and diseased Yesso scallop (*Patinopecten yessoensis*). *AMB Express* 9, 148. <https://doi.org/10.1186/s13568-019-0870-x>.
- Zorz, J., Willis, C., Comeau, A.M., Langille, M.G.I., Johnson, C.L., Li, W.K.W., LaRoche, J., 2019. Drivers of regional bacterial community structure and diversity in the Northwest Atlantic Ocean. *Front. Microbiol.* 10, 281. <https://doi.org/10.3389/fmicb.2019.00281>.