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Changes in bacterial communities in chilled American lobster (*Homarus americanus*) tissues following mortality

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ABSTRACT

Studies evaluating bacteria growing on dead American lobster (*Homarus americanus*) are typically based on a single observation of the post-mortem period. In this study, seventy-five lobsters were euthanized to evaluate changes in microbiological composition between sexes, and in gut, tail, and claw tissues over a 4-day period under chilled (4 °C) storage. The hygienic quality of meat decreased over the storage period but remained acceptable (<6 log CFU/g). Metagenomic analysis showed alpha and beta diversity differed by tissue type throughout storage time. *Photobacterium* spp. Were the primary spoilage bacteria identified in gut (49%) and tail (89%) but were less abundant in the claw (11%). *Photobacterium* spp. Abundance were unchanged in the gut over the sampling period, whereas their proportion increased in the tail (62%) and claw (32%) over the sampling period. Unlike other studies, *Photobacterium phosphoreum* was the predominant species detected. The detection of bacteria not previously reported in American lobster suggest there could be an association between the microbiota in lobster tissues with their diet and/or habitat, and these factors could influence the spoilage bacteria inhabiting these tissues. Findings identify an opportunity to recover tail and claw meat from dead lobsters with a quality acceptable for human consumption.

1. Introduction

In 2020, over 68 M kg of American lobster (*Homarus americanus*) were landed in Atlantic Canada and contributed to approximately one third of the value of seafood exports from Canada (Fisheries & Ocean Canada, 2022). Lobsters are sold preferentially as live animals. Lobsters unfit to survive distribution to markets are sold to processing facilities, and animals perceived to be dead by a rudimentary visual assessment cannot be processed per provincial fishing regulations (Province of Nova Scotia, 2017). Throughout the live lobster supply chain, multiple stressors are encountered including fast trap hauling speeds, rough handling, temperature fluctuations, extended air exposure, fasting, and changes in water quality (Fotedar & Evans, 2011; Basti et al., 2010; Tirloni et al., 2016). These stressors contribute to mortality, and therefore economic losses for lobster harvesters, buyers, processors, and the Canadian economy.

The spoilage of marine products is mediated by microbial activity, yet the post-mortem growth and changes in the microbial community of American lobster is poorly understood. Crustaceans spoil in a manner comparable to fish, but are characterized by distinct tissue compositions, handling practices, storage conditions, and habitats that may influence the microbiota found in their tissues (Zhuang et al., 2020). Notably, the meats of dead American lobster that were shipped live and received <24 h from harvest measured total aerobic counts of 4.5 log colony forming units (CFU)/g, (Tirloni et al., 2016). Comparatively, Atlantic salmon (*Salmo salar*) fillets stored on ice measured lower total aerobic counts (1.5–3.0 CFU/g) 1–2 days post-harvest, which increased to 6 log CFU/g after 14 days of storage (Moretro et al., 2016). Further, specific spoilage organisms (SSOs) in dead lobster meats were composed of *Pseudoalteromonas* spp. And *Photobacterium* spp. (Tirloni et al., 2016), while SSOs in hemolymph from dead American lobster were composed of *Vibrio* spp. And *Photobacterium* spp. (Jung et al., 2021). Other crustaceans develop distinctive SSO after death. Bacterial community assemblages in Norway lobster (*Nephrops norvegicus*) tails stored on ice for 14 days were composed of *Psychrobacter* spp. And *Pseudomonas* spp. (Bekaert et al., 2015), while tails stored at 0 °C for 7 days were

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composed of *Photobacterium* spp. And *Vibrio* spp. (Gornik et al., 2011). Importantly, gut microbiota in lobsters are dynamic based upon feeding habits, feed composition, and geographic location (Lein et al., 2022). A connection relating gut microbiota to the hemolymph has been described for various lobster species (Lein et al., 2022), suggesting that spatial and temporal factors impacting gut microbiota (Holt et al., 2019) could apply to other tissues as well.

Post-mortem changes in microbial community composition within American lobster tissues from the onset of mortality under controlled conditions, has not yet been described. The present study used long-read next-generation sequencing to describe post-mortem changes in the microbial community composition of chilled American lobster tissues. The goal was to assess if chilled (4 °C) lobsters remain of an acceptable quality for human consumption. Specifically, the identity, abundance, and evolution of microbiota found in different tissues of American lobster stored for 4 days were described.

2. Methods and materials

2.1. Lobster handling

Fifty (50) male and twenty-five (25) female American lobsters were sourced from Lobster Fishing Area (LFA) 33 (44 °N/64 °W) in Nova Scotia, Canada. Lobsters were previously size-graded ranging from 650 to 750 g. Animals were collected within 48 h of landing and transferred in expanded polystyrene (EPS) coolers to the Haley Institute of Animal Science and Aquaculture at Dalhousie University (Truro, Nova Scotia). Lobsters were placed in a recirculating seawater system and allowed to acclimate to 4 °C for 14 days in an unfed state to mimic conditions in local lobster holding facilities.

Lobsters were transferred to the Perennia Innovation Centre (Bible Hill, Nova Scotia) in EPS coolers. All live lobsters were euthanized upon arrival by severing the ventral nerve cord with dissecting scissors on the ventral side of the head, below the mandible and above the coxa where the claws join to the cephalothorax. Lobsters were placed in plastic totes with their tails over the edge of the tote to allow hemolymph to drain by gravity for 20 min before animals were transferred into enclosed EPS coolers and held under chilled storage at 4 °C.

2.2. Tissue sampling

Five (5) of each male and female lobsters were removed from chilled storage daily for 4 days, and tail meat, claw meat, and intestinal tissues were aseptically sampled. Two cuts were made originating at the top of the abdomen, 1 cm on either side of the medial line up to the tail fan, and subsequently pulled away from the cephalothorax to reveal the lower intestinal tract. The entire lower segment of the intestinal tract was clamped, excised, and transferred to a sterile sampling jar (Fisher Scientific). A segment of the tail meat, free from contact with the hepatopancreas and/or ovaries and the anus, was separated and transferred to a sterile sampling jar (Fisher Scientific). Claws were separated from the cephalothorax and dissection scissors were used to divide the claw into two segments. The claw meat was separated from the shell using a sterile scalpel then transferred to a sterile sampling jar (Fisher Scientific).

2.3. Aerobic plate counts

Enumeration of total aerobic bacteria in lobster tail and claw tissues was completed using 3M™ Petrifilm aerobic count plates (Innovation Diagnostics Inc., Quebec, Canada) following the supplied protocol. Briefly, sterile tissues were comminuted with sterile scissors until reduced to <1.0 cm in diameter. Ten (10) g of tissue was transferred aseptically into a filter bag (Whirl-Pak, Prosource Scientific Inc., Ontario, Canada), then combined with 90 mL of Butterfield's Phosphate buffer (pH 7.2) and placed in a stomacher (Stomacher 80 Biomaster, Seward, West Sussex, UK) for 2 min on high speed. The sample suspension was transferred into a clean tube and serial dilutions were prepared using Butterfield's Phosphate buffer as the diluent. 3M™ Petrifilm aerobic count plates were placed on a flat surface and 1 mL of sample suspension was dispensed in the centre of the plate. The sample suspension was distributed on the plate using a 3M™ Petrifilm Spreader. 3M™ Petrifilm aerobic count plates were incubated at 35 °C for 48 h ± 3 and all colonies were enumerated regardless of size, colour, or intensity and expressed as log CFU/g. Enumeration of total bacteria for each tissue was performed in duplicate.

2.4. Genomic DNA extraction

Extraction of genomic DNA from lobster tissues was performed using the DNeasy PowerFood Microbial Kit (Qiagen, Hilden, Germany) following the protocol provided by the manufacturer, with modifications. Briefly, lobster tissues were comminuted with sterile scissors until reduced to <1.0 cm in diameter. Tissues were combined with sterile phosphate buffered saline (PBS) at a ratio of one part tissue to 3 parts PBS, then placed in a stomacher (Biomaster) for 120 s on high speed. A 1.5 mL aliquot of sample suspension was transferred to a 2 mL microcentrifuge tube and then centrifuged at 13,000×*g* for 5 min (Sorvall ST8, ThermoFisher Scientific, Waltham, Massachusetts) at ambient temperature. The supernatant was aspirated using a pipette tip to remove as much liquid as possible without disturbing the pellet, and DNA extraction followed the protocol provided by the manufacturer (DNeasy PowerFood, Qiagen). An additional ethanol washing step to improve the purity of the DNA extracts was completed. The extracted DNA was assessed for concentration and purity by spectrophotometer (Model DS-11, DeNovix, Wilmington, DE) at 230 nm, 260 nm, and 280 nm. DNA extracts were stored at −20 °C until further analysis.

2.5. Full length 16 S rRNA gene sequencing

Metagenomic sequencing was performed by the Integrated Microbiome Resource (IMR) testing lab at Dalhousie University following PacBio protocol 101-599-700. Preparation of amplicon libraries was completed following the protocol by Comeau and Filloramo (2023).

2.6. Texture analysis

Five (5) dead male lobsters at each post-mortem interval were cooked by submerging in boiling water for 10 min and submerged in an ice slurry for an additional 15 min following cooking. Lobsters were removed from the ice slurry and the tails were separated from the cephalothorax. The dorsal side of the tail shell was cut using dissection scissors along the medial line, followed by an identical cut along the ventral side of the to separate the tail meat. The two lobes of the tail were separated using a knife, and the second segment from each lobe was excised and used for texture analysis. Texture measurements were completed using a texture analyzer (TA.XT.2, Stable Micro Systems, Surrey, UK) following a modified method of Humaid et al. (2019). A texture profile analysis (TPA, n = 5) test was performed with a 2 kg load cell, using a TA-25 platen at 1 mm/s test speed. Two compressions to 50% strain were applied with a 5 s gap between compressions.

2.7. Analysis

Changes in microbiota present in post-mortem American lobster tissues were investigated by sequencing full length 16 S rRNA gene sequences. Bioinformatics were performed with QIIME 2 version 2017.6 (Bolyen et al., 2019), as previously described (Pearson et al., 2019). The amplicon sequence variants (ASV)-level taxonomic composition in lobster tissues were analyzed using QIIME2, with modifications to filter bacterium at < 1% overall sample abundance. The STAMP v2+ software package was used to analyze differences in the taxonomic profiles between groups and pairs of samples (Parks et al., 2014). Principal

component analysis (PCA) was used to explain the variation of weighted Unifrac, Bray-Curtis, and Jaccard dissimilarity matrices. R Version 4.2.1 (R Core Team 2022) and the 'vegan' (Oksanen et al., 2022) package were used to assess differences in the bacterial assemblages with the ANOSIM and PERMANOVA functions. Non-metric multi-dimensional scaling (NMDS) plots were generated to show the clustering of samples. Exponent 32 software (Stable Micro Systems, Surrey, UK) was used to calculate the individual sample heights and TPA parameters of hardness, springiness, chewiness, gumminess, cohesiveness, and resilience (Friedman et al., 1963). ANOVA was used to test for differences in textural parameters between sample groups and bacterial loads within all samples. Welch's unequal variance *t*-test was used to test for differences in taxonomic composition. All statistical tests used a significance level of $\alpha = 0.05$.

3. Results

3.1. Bacterial counts

There were no significant differences in total aerobic bacterial counts between lobster tissue types ($p = 0.81$) or sexes ($p = 0.18$) over the post-mortem storage period, however, there were significant differences in counts over this period ($p < 0.001$) (Fig. 1). Tukey's post-hoc showed no significant differences in bacterial counts between Days 0–2 ($p > 0.05$). Bacterial counts on Day 4 were significantly greater than all previous sampling days ($p < 0.001$). Further, bacterial counts on Day 3 were significantly higher than all previous days ($p = 0.04$) except for Day 2 ($p > 0.05$). Gut tissues were not assessed for total aerobic bacterial counts.

4. Bacterial diversity

4.1. Summary of sequencing

Generation of an amplicon sequence failed in 20% of samples (30/150), and no significant differences ($p > 0.05$) were identified between tissue types, sexes, or sampling periods within these failed samples. A total of 1,081,051 ASVs were retained across 120 samples after DADA2 quality filtering, denoising, and removal of non-chimeric sequences. Sample reads ranged from 1085 to 48,269 per sample (Supplementary Table 1).

4.2. Alpha diversity

After DADA2 filtering, 3330 operational taxonomic units (OTUs) were described in total ($n = 120$), ranging from 24 to 255 within individual samples (Supplementary Table 2). From 1272 to 1448 OTUs were identified within different tissue types, compared to 1937–2373 OTUs between sexes, and 977–1358 OTUs at different sampling intervals. Between 40 and 50% of OTUs were identified in common across sexes (Fig. 2A) compared to 9–10.5% of OTUs between tissue types (Fig. 2B), and 13–19% of OTUs between all sample intervals (Fig. 2C). Average Shannon Diversity Index scores ranged from 4.79 to 5.23 across all categories and from 2.47 to 6.86 within individual samples. There were no significant differences in Shannon Diversity Index score between sexes ($p = 0.77$) or sampling intervals ($p = 0.78$), however, there were significant differences between sample types ($p < 0.1$). Kruskal-Wallis pairwise post-hoc tests identified significant differences between the gut and tail tissues ($p < 0.05$) (Supplementary Table 2).

4.3. Beta diversity

Primary and secondary axes on PCA plots based on weighted Unifrac, Bray-Curtis, and Jaccard dissimilarity, explained 67.09% (PC1 = 50.99%, PC2 = 16.10%), 26.77% (PC1 = 18.28%, PC2 = 8.49%), and 14.04% (PC1 = 9.28%, PC2 = 4.76%), respectively, of variation in these samples (Supplementary Fig. 1).

Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity showed clustering of OTUs by tissue type, but not by sex or sample interval (Fig. 3). These results were supported by PERMANOVA, where significant differences based on sample type ($R^2 = 0.16$, $p < 0.001$), sample interval ($R^2 = 0.05$, $p < 0.001$), the interaction between sample type and sample interval ($R^2 = 0.09$, $p < 0.001$), and sex ($R^2 = 0.01$, $p < 0.05$) were identified. Pairwise tests between sample types indicated that the sample type, sample interval, and their interaction were significant factors contributing to differences in beta-diversity between each sample type pairing (Table 1). Additionally, sex was a significant factor contributing to the dissimilarity between tail tissues with both gut ($p < 0.1$) and claw ($p < 0.05$) tissues, but not between gut and claw alone.

A cluster dendrogram based on Bray-Curtis dissimilarity (Fig. 4) was generated using PERMANOVA and branches indicate a distinction in the beta-diversity between different tissue types. In all sample types, contiguous days are closely related, whereas samples taken at the beginning of the sampling period were less likely to be clustered with

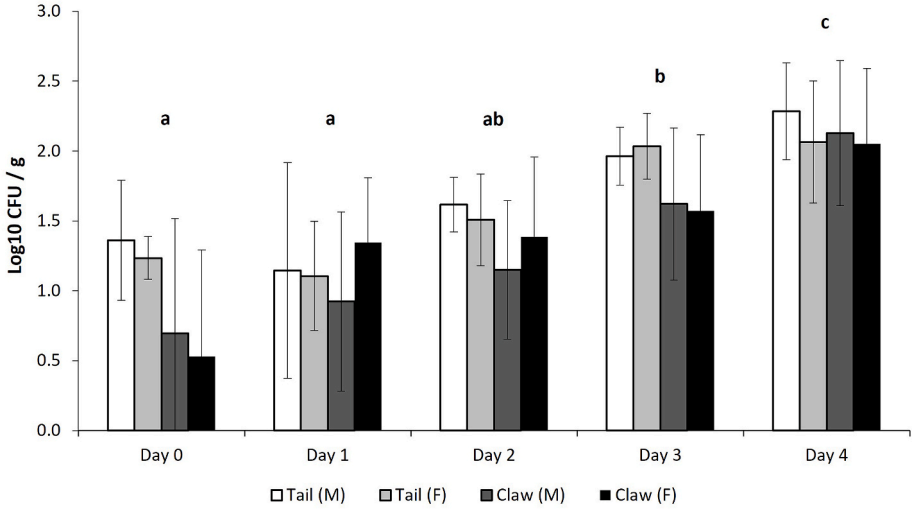

Fig. 1. Total aerobic bacteria in American lobster (*Homarus americanus*) meat tissues over the 4 d sampling period using 3 MTM Petrifilm aerobic count plates. Lowercase letters indicate significant differences ($p < 0.05$) between sampling intervals when comparing all samples, following Tukey's post-hoc test.

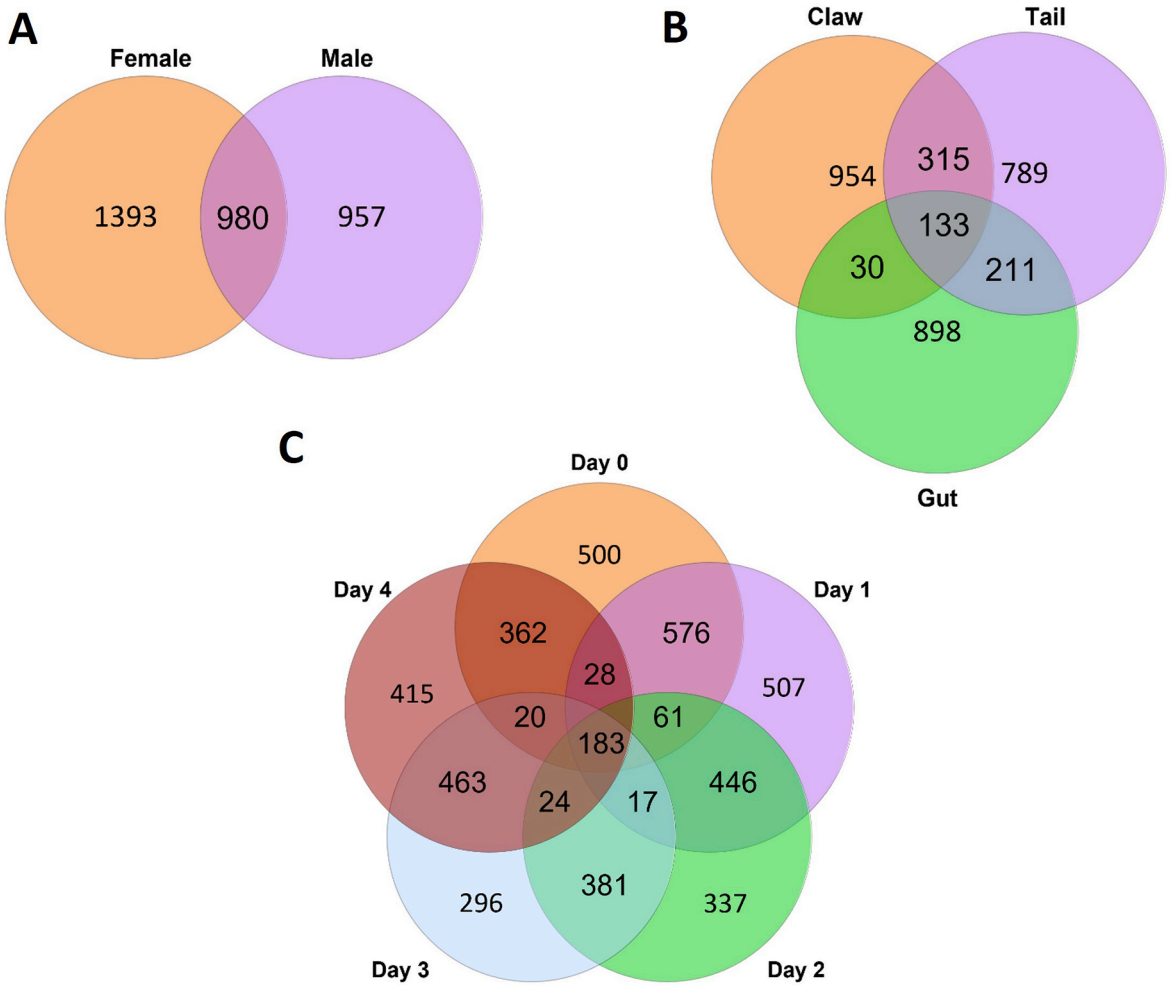

Fig. 2. Distribution of unique and non-unique operational taxonomic units (OTUs) within American lobster (*Homarus americanus*) tissues by sample groupings by (A) sex, (B) tissue type, and (C) sampling interval.

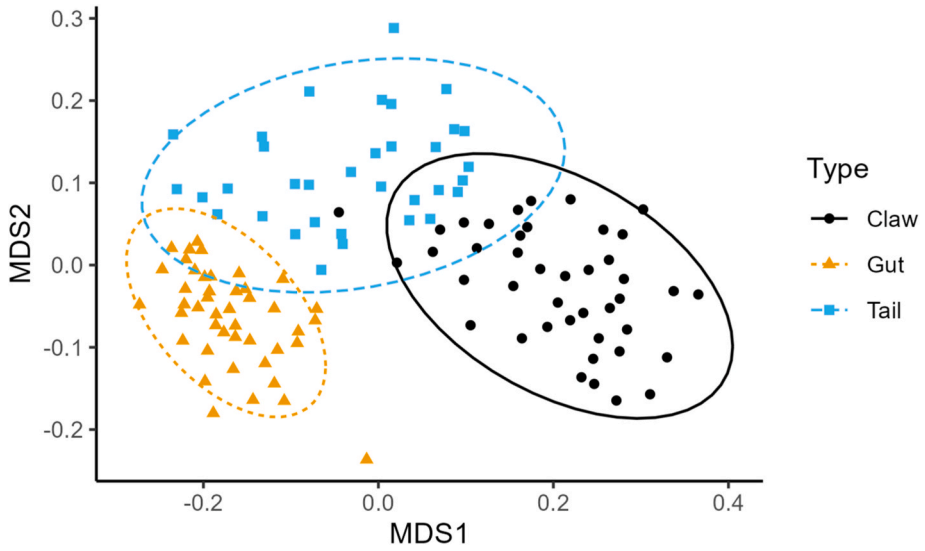

Fig. 3. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity of bacterial communities in different American lobster (*Homarus americanus*) tissue types.

Table 1
Post-hoc PERMANOVA pairwise comparisons between tissue types for factors affecting beta-diversity.

Pairings	Variable	R^2	F	p-value
Tail vs Claw	*Type*	0.094	8.635	0.001
	Interval	0.082	1.860	0.001
	Sex	0.017	1.591	0.023
	Type x Interval	0.083	1.893	0.001
	Type x Sex	0.011	0.969	0.466
	Interval x Sex	0.051	1.169	0.096
Tail vs Gut	*Type*	0.096	8.736	0.001
	Interval	0.065	1.486	0.005
	Sex	0.015	1.399	0.087
	Type x Interval	0.076	1.721	0.001
	Type x Sex	0.010	0.932	0.534
	Interval x Sex	0.051	1.157	0.148
Claw vs Gut	*Type*	0.178	19.531	0.001
	Interval	0.064	1.749	0.001
	Sex	0.011	1.173	0.220
	Type x Interval	0.056	1.531	0.005
	Type x Sex	0.011	1.155	0.230
	Interval x Sex	0.040	1.091	0.227

those taken at the end. Additionally, lobster tails on Days 3 and 4 showed greater similarity to the gut on Days 3 and 4 than to tails sampled on prior days.

4.4. Taxonomic composition

A total of 8 phyla were identified using the ASV-level taxonomic composition (Fig. 5A). *Proteobacteria* were detected in comparable proportions in female (58.5%) and male (57.5%) lobster, predominated tail tissues (97.7%), and increased in abundance two-fold from 42% to 81.7% from Day 0 to Day 4 across all tissue types.

A total of 26 genera were identified (Fig. 5B). *Photobacterium* spp. Were identified in comparable proportions in female (51%) and male (48%) lobster, predominated tail tissues (89%), and increased in abundance two-fold from 37% to 70% from Day 0 to Day 4 across all tissue types.

4.5. Changes in taxonomic composition by sex

In tail tissues, Welch's *t*-test of species-level taxa determined an unclassified *Pseudoalteromonas* was significantly more abundant in females (7.64% ± 10.63%) than males (1.58% ± 2.42%) ($p < 0.05$), and an unclassified *Photobacterium* was significantly more abundant in males (7.37% ± 4.21%) than females (4.51% ± 2.55%) ($p < 0.05$). No significant differences ($p > 0.05$) were detected in claw or gut tissues.

4.6. Changes in taxonomic composition by tissue type

At the phylum-level (Fig. 6A), *Proteobacteria* was more abundant in tail tissues compared to other tissue types by a proportion of 40% ($p < 0.001$), whereas claw tissues had significantly higher abundances of *Campilobacterota* (31%; $p < 0.001$) and *Desulfobacterota* (22%; $p < 0.001$), and gut tissues had significantly higher abundances of *Fusobacteriota* (21%; $p < 0.001$) than other tissues.

At the genus-level, *Photobacterium* was more abundant in tail tissues compared to all other tissues ($p < 0.001$) (Fig. 6B). Claw tissues contained significantly higher proportions of *Sulfurospirillum, Sulfurovum,* and others ($p < 0.01$), and gut tissues contained significantly higher proportions of *Photobacterium and Psychrilyobacter,* and others ($p < 0.01$), compared to all other tissues.

4.7. Changes in taxonomic composition by sample interval

Bacteria detected at > 1% abundance based on ASV-level taxonomy and that changed significantly ($p < 0.05$) in abundance over the sampling period were identified (Table 2) following an ANOVA with Tukey-Kramer post-hoc testing. No significant genus-level taxonomic changes in gut tissues were identified over the sampling period ($p > 0.05$). The taxa changing in absolute abundance over the sampling period are represented across individual tissue types (Fig. 7). A significant increase in abundance of *Photobacterium* was found when all tissues were evaluated together ($p < 0.001$), including tail and claw tissues alone, but not the gut. A similar increase was also observed by *Photobacterium phosphoreum* ($p < 0.01$), which reached an abundance of 75% in tails ($p < 0.001$), but only 17% in the claws ($p < 0.05$) and did not change in abundance within the gut over the sampling period. The genus *Moritella* increased in abundance specifically in the claw ($p < 0.001$), whereas *Psychrilyobacter* decreased in abundance specifically in the tail ($p < 0.05$). Decreases in the proportion of phyla *Desulfobacterota* ($p < 0.001$), and *Campilyobacter* ($p < 0.1$) were observed in the claw tissues over the sampling period.

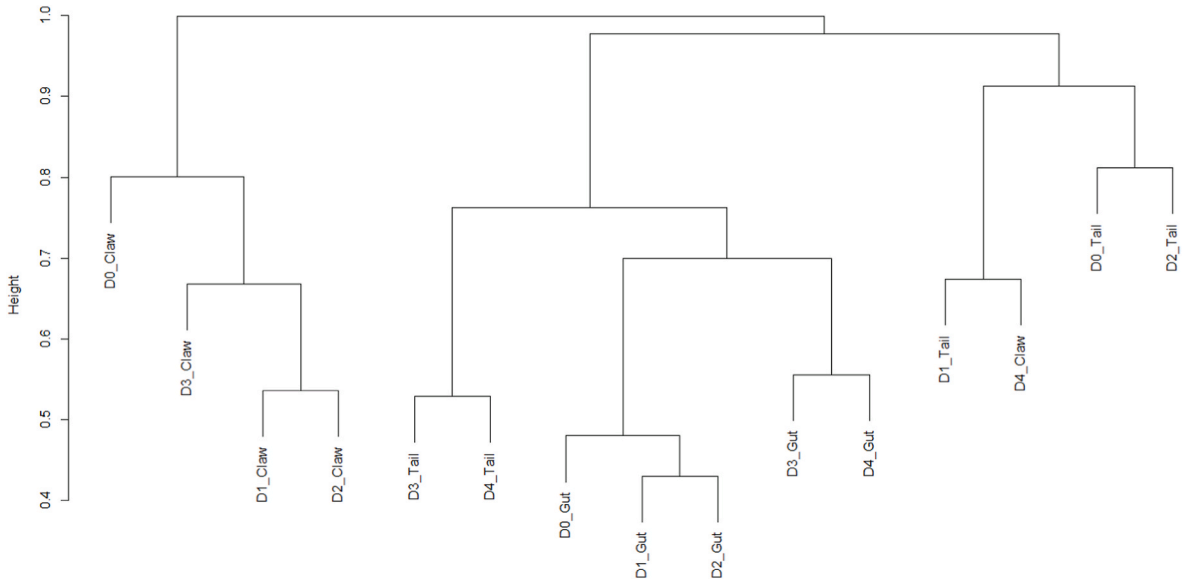

Fig. 4. Cluster dendrogram based on Bray-Curtis dissimilarity for microbiota identified within all American lobster (*Homarus americanus*) samples collected over the experimental period.

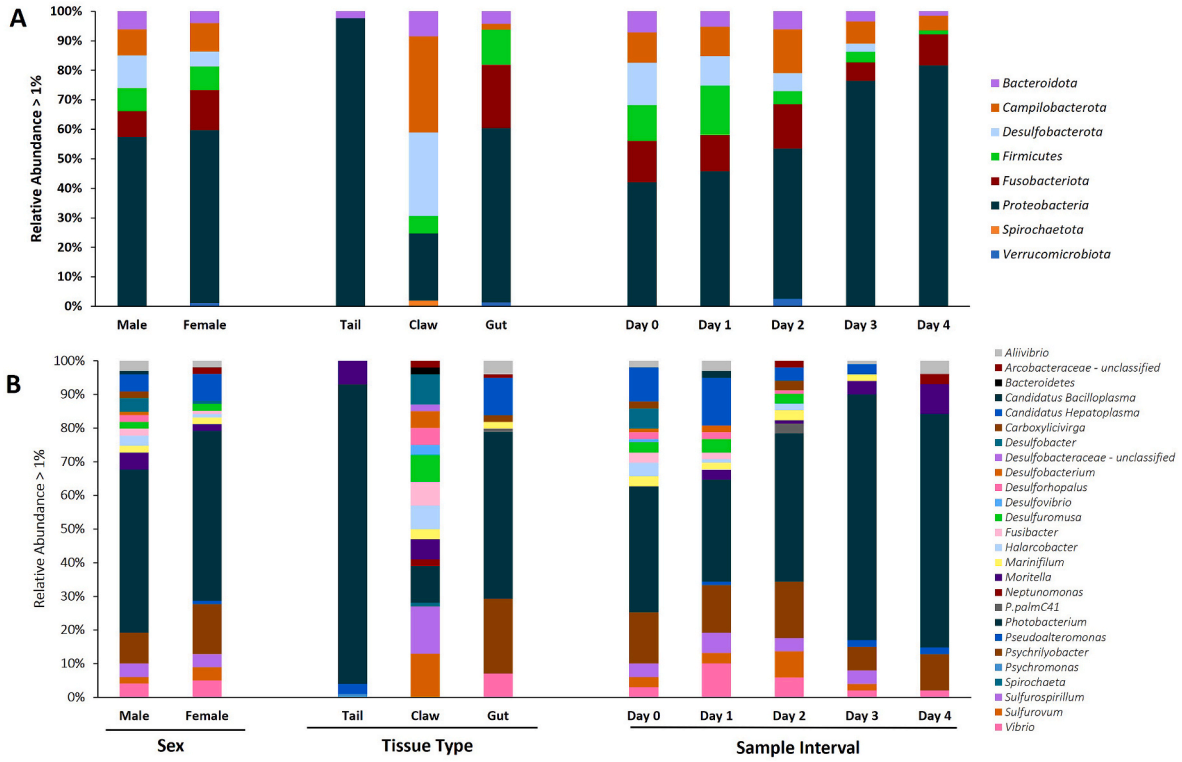

Fig. 5. Taxonomic composition of bacteria identified within sample groups at > 1% relative abundance at the level of (A) phylum and (B) genus.

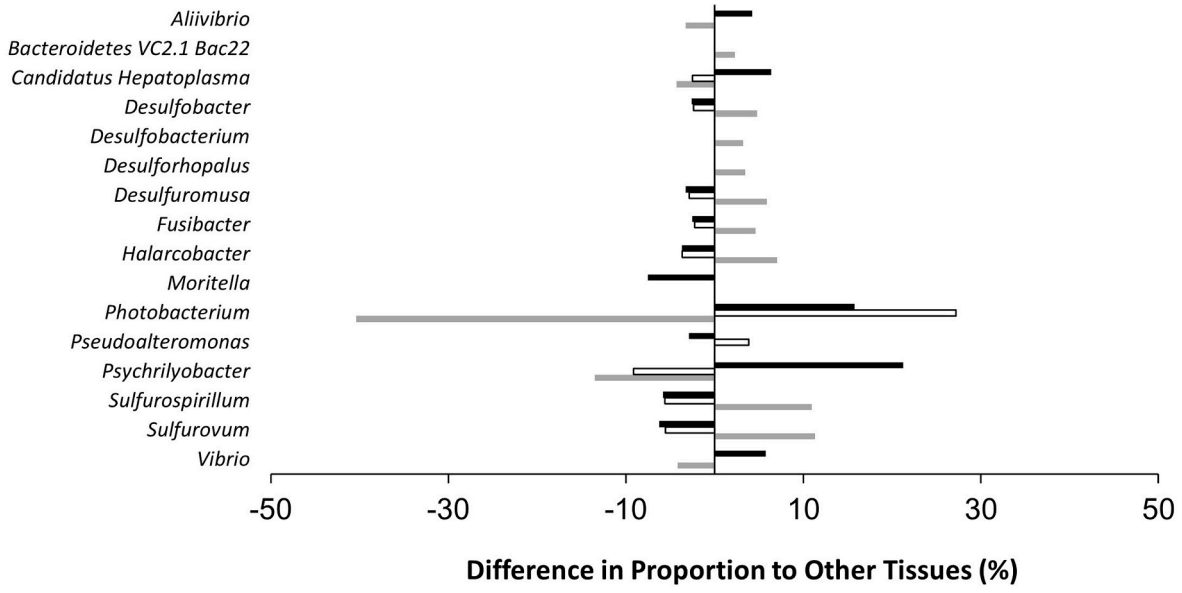

Fig. 6. Bacterial taxa at (A) phylum, and (B) genus levels that were significantly different ($p < 0.05$) within each tissue type relative to all other tissues following a Welch's unequal variance test and that changed >2% in the overall proportion.

4.8. Texture analysis

Following cooking, "Mushy tail" (Fig. 8B), where meat showed poor adhesion to the shell and segments of the flexor muscle were weakly attached to each other, was detected in 20% of replicates on Day 1 and increased to 40% on Days 2–4. Excluding these samples resulted in unequal sample sizes and affected the analysis of the results (Fig. 8A). ANOVA on TPA parameters including mushy samples revealed a significant decrease in resilience ($p < 0.1$) over the sampling period. No other trends were observed in the other texture attributes (Table 3). Post-hoc tests indicated resilience in cooked lobster tails were significantly different ($p < 0.05$) between Day 0 and Day 4.

5. Discussion

In this study, meta-genomic sequencing was used to describe the microbial changes in chilled, post-mortem American lobster tissues. Specific spoilage organisms were characterized in the edible meat components (tail and claw), from the onset of mortality to olfactory rejection, and texture quality attributes were used to describe how tissue

Table 2
Genus-level microbiota detected at > 1% relative proportion exhibiting significant changes in abundance within lobster tissues (p-value <0.05) over the sampling period determined using ANOVA with post-hoc Tukey-Kramer (n = 50).

	Claw		Tail	
Genus	Change in Abundance (%)	p-value	Change in Abundance (%)	p-value
Aliivibrio	1.45	0.0254	–	–
Arcobacteraceae - unclassified	6.19	0.0052	–	–
Candidatus Hepatoplasma	–	–	9.06	0.0379
Carboxylicivirga	–	–	1.30	0.0248
Cardiovacteriaceae - unclassified	–	–	1.42	0.0012
Cocleimonas	–	–	1.91	0.0085
Cohaesibacter	1.54	8.08×10^5	–	–
Colwellia	2.77	0.0128	–	–
Desulfovibrio	3.22	7.72×10^5	–	–
Desulfuromusa	10.09	2.12×10^5	–	–
Draconibacterium	0.99	0.0314	–	–
Flavobacteriaceae - *unclassified*	1.14	0.0455	1.99	0.0001
Fusibacter	5.86	0.0033	–	–
Leucothrix	–	–	1.24	0.0217
Litoreibacter	–	–	1.25	0.0064
Methylovorus	–	–	9.34	0.0112
Moritella	24.07	6.01×10^7	–	–
Photobacterium	31.42	0.0028	62.06	0.0018
Pseudahrensia	–	–	3.07	8.46×10^5
Pseudoalteromonas	4.38	0.0022	–	–
Psychrilyobacter	–	–	17.82	0.0381
Rhodobacteraceae - *unclassified*	–	–	3.29	1.92×10^5
Rubritalea	–	–	3.49	0.0005
Saprospiraceae - unclassified	–	–	1.84	0.0001
Shewanella	–	–	1.53	0.0264
Sulfurospirillum	18.33	0.0208	–	–
Thiothrix	–	–	1.06	3.35×10^5
Vibrio	–	–	3.68	0.0336

quality changed over this period. Changes in the microbiota of typical seafood products post-mortem first undergo an initial loss of biodiversity, with SSOs becoming the predominant microbiota prior to sensory rejection (Zhuang et al., 2020). Following this, the proportions of SSO continue to fluctuate beyond the sensory rejection point (Zhuang et al., 2020). After 4 days of refrigerated storage of American lobster in the present study, persistent sour odours were detected surrounding the cephalothorax of the whole lobster, and internal organs had begun to liquify, indicating spoilage had progressed considerably in these animals. Total aerobic counts in claw and tail tissues (<2.1 log CFU/g) were below the threshold for rejection based on the standard bacterial load in seafood products of 6 log CFU/g (Kim et al., 2016). These values were lower than previously described in the meats of dead American lobster sampled <24 h post-harvest (Gornik et al., 2011; Tirloni et al., 2016). The differences may be attributed to a unique ecological environment in the live holding facility where animals were kept prior to euthanasia (Holt et al., 2019), or by how animals were handled once removed from water storage (Humaid et al., 2019; Jung et al., 2021), as live lobster are susceptible to microbial invasion ante-mortem.

In this study, textural changes were observed in post-mortem lobster tails. Evidence of mushy tail, as previously reported by Calder et al. (2005), was detected with increasing frequency over the storage period, and the release of proteolytic enzymes from the hepatopancreas was the suspected cause (Marshal et al., 1987). In contrast, softening of fish muscle post-mortem occurs due proteolytic enzymes acting on myofibrillar protein networks within muscle cells (Huss, 1995). Hardness, the TPA parameter used to describe the extent of this softening (Cheng et al., 2014), was unchanged over the storage period. Conversely, resilience, a TPA parameter indicating the force made by the sample to return to its original position after compression, decreased slightly over the sampling period. High Pressure Processing (HPP) has been shown to affect the structure of myofibrillar protein networks (Yagiz et al., 2009), yet both hardness and resilience were unchanged following HPP of American lobster tails (Humaid et al., 2019). Any changes that did occur in the myofibrillar protein network during the storage period did not result in significant changes to the physiochemical attributes of the lobster tai in the present study. Lobster tails are complex arrangements of muscle fibre bundles, and changes in resilience over the storage period may be mediated by changes to stromal proteins that provide structure between discrete muscle fibre bundles. Notably, evidence of “mushy tail” could not be identified in the raw state and has been suspected to only be present after cooking dead lobster. This theory is supported by observations in the present study from samples after only 1 day of post-mortem storage. Overall, post-mortem American lobster is vulnerable to both microbial and enzymatic degradation, but further characterization of the post-mortem biochemistry within these tissues is needed to improve the understand of these mechanisms.

Indicators of alpha and beta diversity revealed distinct microbiota within each tissue type, and independent analysis of each tissue type described a unique progression of microbiota over the post-mortem storage period in the present study. Common SSO detected in fish and crustaceans include *Aeromonas*, *Photobacterium*, *Pseudomonas*, and *Shewanella* (Zhuang et al., 2020). Conversely, the data in the present study indicated the phyla *Proteobacteria* characterized the primary SSOs within all American lobster tissues. Species-level identification indicated *Photobacterium phosphoreum* as the predominant SSO in tail and claw tissues.

Photobacterium phosphoreum is a gram-negative, psychrotolerant, facultative aerobe that can decompose trimethylamine *N*-oxide (TMAO) to trimethylamine (TMA) and can participate in the production of histamine which has been detected within vacuum and modified atmosphere packaged finfish (Dalgaard et al., 1993; Dalgaard et al., 1997). *Photobacterium phosphoreum* was not previously identified in tissues of dead American lobster (Jung et al., 2021; Tirloni et al., 2016), but was identified in Norway lobster tails (Gornik et al., 2011). Amplicon sequence variants of other *Photobacterium* spp. Detected within tail tissues included *P. profundum* and *P. iliopiscarium,* that were also not previously described in the meats (Tirloni et al., 2016) or hemolymph (Jung et al., 2021) of dead American lobster. Following storage of Norway lobster tails for 7 days at 0 °C, *P. profundum* was detected at an equal proportion to *P. phosphoreum* (Gornik et al., 2011), but this was not observed in the present study. Other common SSO such as *Pseudomonas* and *Shewanella* were previously detected within dead American and Norway lobster meats (Bekaert et al., 2015; Tirloni et al., 2016), but were not detected in any samples in the present study. Additionally, *Vibrio* spp. Were detected in relatively high abundance in both American (17.5%) and Norway (40%) lobster in post-mortem tissues (Gornik et al., 2011; Jung et al., 2021), whereas *Vibrio* peaked at 10% in the gut and tail on Day 1 and decreased thereafter in the present study. *Vibrio* was not identified in claws in the present study.

Microbiota inhabiting the gut of crustaceans are influenced by food availability, diet composition, and habitat (Lein et al., 2022). Gut bacteria in this study were unchanged in composition and relative abundance over the post-mortem storage period. As lobster were unfed for at least 14 days prior to experimentation, and with others reporting a highly variable gut microbiome over a 24 h period in Chinese mitten crab (Yu et al., 2021), the microbiota detected within the gut may have already undergone compositional changes and reached an equilibrium prior to euthanasia and the start of the experimental period. The gut

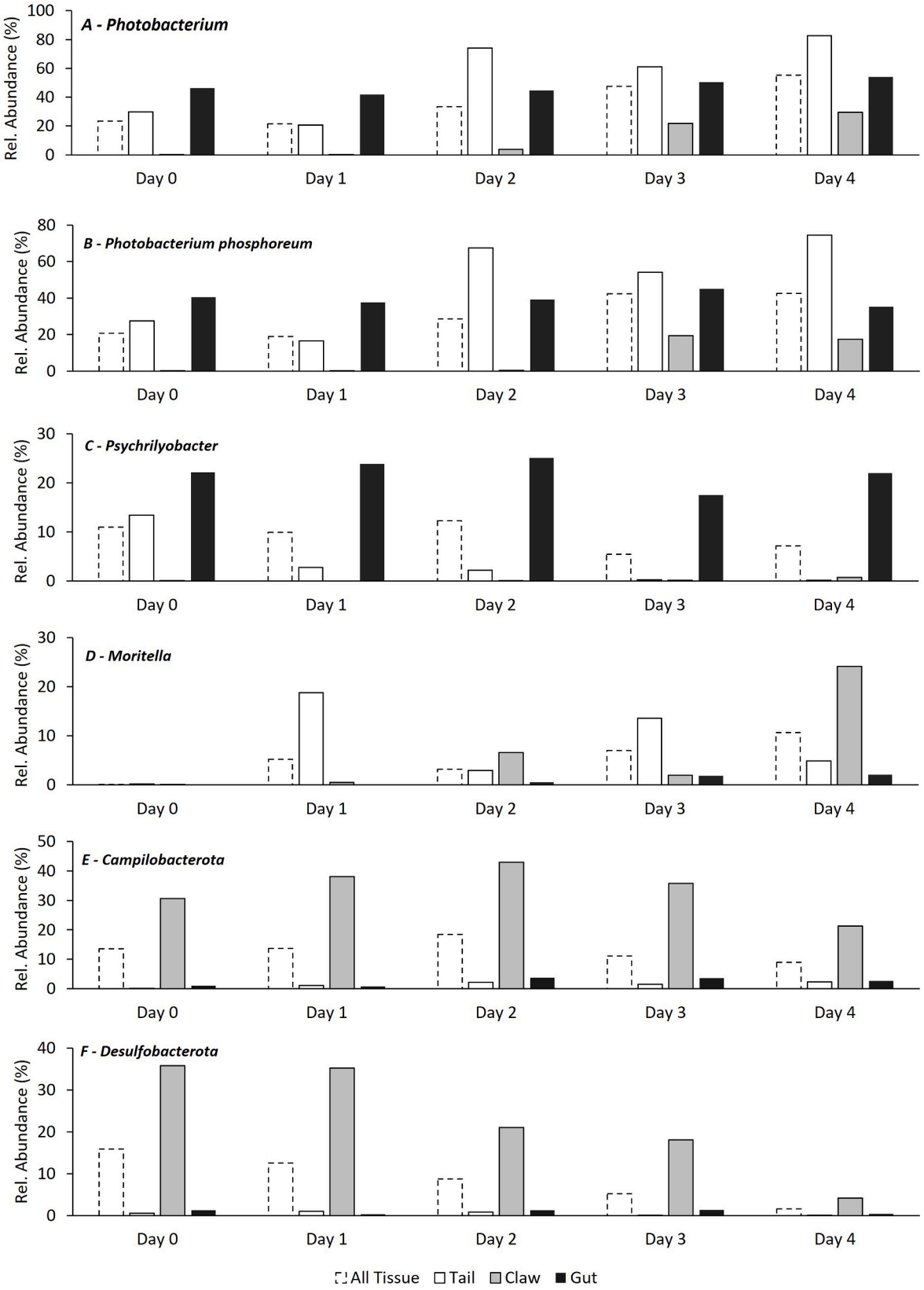

Fig. 7. Changing proportions of (A) the genus *Photobacterium*, (B) *Photobacterium phosphoreum*, (C) the genus *Psychrilyobacter*, (D) the genus *Moritella*, (E) the phyla Campilobacterota, and (F) the phyla Desulfobacterota in each tissue and overall, over the sampling period. Relative proportions are filtered to remove taxa found at < 1% overall abundance.

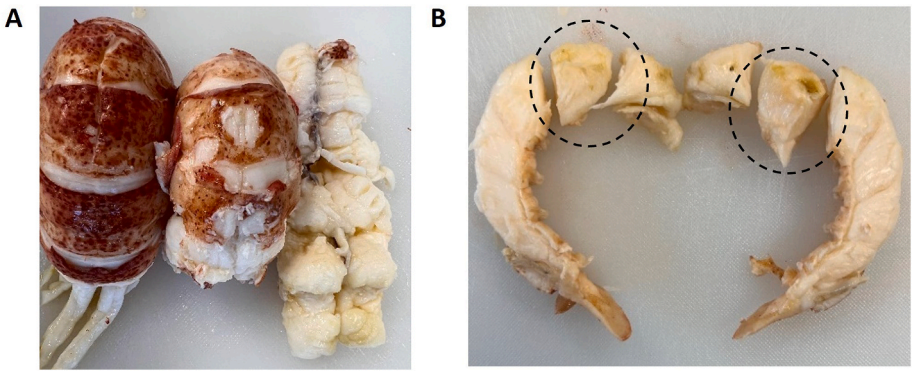

Fig. 8. Cooked American lobster (*Homarus americanus*) tails used in texture analysis. (A) Examples of mushy tail, Left: unaffected, Middle: slightly affected, right: severely affected. (B) Preparation of lobster tail for texture analysis with circles indicating which segment was used.

Table 3
Texture profile analysis (TPA) of cooked post-mortem male lobster tail (n = 5). Letters indicate significant differences (p-value <0.05) by ANOVA with post-hoc Tukeys HSD test.

	Day 0		Day 1		Day 2		Day 3		Day 4	
Parameter	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Hardness	2084.15	691.42	1910.16	806.43	2062.52	807.63	2183.84	303.76	1870.90	988.70
Resilience	28.09[a]	2.32	25.90[a]	2.75	25.06[a]	1.87	25.85[a]	1.93	23.72[b]	6.03
Cohesiveness	0.55	0.03	0.54	0.03	0.51	0.02	0.54	0.04	0.53	0.07
Springiness	60.94	6.58	68.14	8.41	63.62	6.90	65.46	4.61	60.84	8.07
Gumminess	1148.34	381.42	1036.82	438.19	1062.96	424.91	1166.95	139.31	1025.08	586.92
Chewiness	723.54	287.81	724.01	331.12	693.11	351.81	764.78	108.99	658.06	421.96

microbiome was initially composed of high proportions of *P. phosphoreum* (>40%), and of *Psychrilyobacter* and *Candidatus* hepatoplasma that were each detected exclusively in the gut. These results are consistent with other studies (Lein et al., 2022; Ooi et al., 2017). *Psychrilyobacter* are gram-negative obligate anaerobes (Zhao et al., 2009) with strong proteolytic capabilities (Pelikan et al., 2021), and *Candidatus Heptoplasma* has also been identified in the gut of Norway lobster (Meziti et al., 2012), but unlike these genera, *P. phosphoreum* is a known SSO that can proliferate outside of the gut environment (Dalgaard et al., 1993). These findings may suggest a relationship between lobster bait and gut bacterial composition.

The claw microbiome was characterized by both significant increases and decreases in bacterial taxa. Initially, claws possessed high abundances of the phyla *Desulfobacterota* and *Campilobacterota* that were greatly reduced by Day 4. Bacteria of these phyla possess sulfate reducing and sulfur oxidizing functions (Carrier et al., 2020), respectively, and serve important functions of maintaining the geochemical sulfur cycle. These bacteria often associated with deep-sea hydrothermal vents and anoxic substrates in the marine environment (Begmatov et al., 2021), and have even been detected within gut and gill tissues of the deep-sea hydrothermal vent crab (*Austinograea* sp.) (Zhang et al., 2017). The identification of these may suggest that microbiota detected within claws provide evidence of lobster habitat. As with *P. phosphoreum, Moritella* was initially undetected within claw tissues in the present study and both reached a comparable proportion by the end of the storage period (29.1% vs. 24.1%, respectively). *Moritella* were not previously identified in tissues of dead American or Norwegian lobster. *Moritella* are psychrophilic and piezophilic facultative anaerobes that have also been detected in the gut of deep-sea hydrothermal vent crabs (Zhang et al., 2017). *Moritella* also did not show growth in either gut or tail tissue, so their proliferation in claws may be driven by lower competition with other spoilage organisms, or by the availability of unique nutrients. This could also indicate an influence of habitat on the spoilage of lobster tissues.

The influence of sex on gut microbiomes has been previously described in humans (Kim et al., 2016) and Caribbean spiny lobster (*Panulirus argus;* Zamora-Briseno et al., 2020). Sex differences have not been previously described for spoilage bacteria of American lobster; however, this may be an artefact introduced by higher PCR failure rates in the tail tissues, male lobsters, and earlier sample intervals. *Pseudoalteromonas* and *Photobacterium* were previously detected in high abundance in the meats of dead American lobster (Tirloni et al., 2016) yet, at the end of the experimental period in this study lobster meats possessed low bacterial counts and high proportions of different *Photobacterium* spp. This may indicate the storage period captured only the first phase of spoilage involving SSOs becoming the dominant taxa (Zhuang et al., 2020). Thus, a longer sampling period could affirm these sex differences or render them undetectable, as SSO are expected to continually evolve with extended storage.

The microbiome from lobster shell is compositionally distinct from the microbiome from lobster hemolymph, and it was suspected that the hemolymph was populated by bacteria migrating through the gut (Jung et al., 2021). This conjecture that bacterial contamination of American lobster moves through the gut is supported by results in this study where the abundance of *P. phosphoreum* in the gut is high and unchanged over the sampling period, but steadily increases in both tail and claw tissues. Further, a stronger association between gut and tail microbiomes was indicated by the number of shared OTUs between these tissues, and by the stronger similarity in beta-diversity, compared to the gut and claw. Lobsters used in this study differed from previous studies evaluating the post-mortem lobster microbiome (Jung et al., 2021; Tirloni et al., 2016) by their harvest location. As both diet and habitat are known factors that influence the gut microbiome, SSO detected in lobster tissues would be expected to vary with food availability, quality, and/or composition. The gut microbiota in captive Norway lobster were found to be unrelated to the bacteria detected in the water or their diets, but the nutrient composition of their diet did inform the selection of specific microbial species found within the guts of the natural population (Meziti et al., 2012). Future studies should be completed to evaluate how diet and nutrition select for microbiota within the gut of American lobster, and if different SSOs impact the overall spoilage progression of raw lobster products or within different tissues.

Overall, these results show, when dead lobsters are maintained at chilled temperatures, there is a limited window when the microbiological and physical attributes of the edible meat components of American lobster remain adequate for human consumption. There may be an opportunity to establish process controls for the recovery of edible meat for commercial sale without the risk of producing decomposed or unwholesome product, or product tainted by chemical hazards (i.e., histamine), if storage temperatures and the duration since confirmed alive can be verified in these animals.

6. Conclusions

This study evaluated the post-mortem changes in American lobster under controlled conditions to understand the development of spoilage bacteria and changes in meat quality over a short storage period. *Photobacterium phosphoreum* predominated both gut and tail tissues over the storage period, but the microbiota detected in different tissues were distinct. Tail meats maintained acceptable textural and microbiological quality over the storage period; however, mushiness was detected in these tissues unpredictably, highlighting both the opportunity and risk of utilizing these animals for commercial application. Data indicate the microbiota found within internal structures was derived from the gut microbiome, and reflected the diet and habitat of these animals that could differentiate harvest areas more than previously thought. A further understanding of the influence of diet and habitat on the bacteria found in American lobster, alive or dead, could produce opportunities to identify lobster bait products that limit spoilage in these animals.

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Conflict of interest and authorship conformation form

Please check the following as appropriate:

X All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

X This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

X The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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Jonathan Rolin: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Funding acquisition. **Ryan A. Horricks:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Jancy Stephen:** Investigation. **Kiersten Watson:** Resources, Writing – review & editing, Project administration, Funding acquisition. **K. Fraser Clark:** Methodology, Resources, Writing – original draft, Writing – review & editing. **Leah M. Lewis-McCrea:** Resources, Writing – review & editing, Project administration, Funding acquisition. **Gregor K. Reid:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115136.

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