The microbial composition of dried fish prepared according to Greenlandic Inuit traditions and industrial counterparts

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A B S T R A C T

The practices of preparing traditional foods in the Arctic are rapidly disappearing. Traditional foods of the Arctic represent a rarity among food studies in that they are meat-sourced and prepared in non-industrial settings. These foods, generally consumed without any heating step prior to consumption, harbor an insofar undescribed microbiome. The food-associated microbiomes have implications not only with respect to disease risk, but might also positively influence host health by transferring a yet unknown diversity of live microbes to the human gastrointestinal tract. Here we report the first study of the microbial composition of traditionally dried fish prepared according to Greenlandic traditions and their industrial counterparts. We show that dried capelin prepared according to traditional methods have microbiomes clearly different from industrially prepared capelin, which also have more homogenous microbiomes than traditionally prepared capelin. Interestingly, the locally preferred type of traditionally dried capelin, described to be tastier than other traditionally dried capelin, contains bacteria that potentially confer distinct taste. Finally, we show that dried cod have comparably more homogenous microbiomes when compared to capelin and that in general, the environment of drying is a major determinant of the microbial composition of these indigenous food products.

1. Introduction

In the tailwind of the enormous focus on the human gut microbiome there is a high interest in the microbial composition of our foods, i.e. the food microbiome (Lang et al., 2014). Our diet impacts our intestinal microbiome not only through the micro- and macronutrients of the food but also through the transfer of microbes from the food to the intestines (David et al., 2014) and these food-associated commensal microbes might be one source of microbes positively influencing human health. Traditional lifestyles among indigenous populations have been coupled with a more diverse and potentially healthier gut microbiome (Clemente et al., 2015; De Filippo et al., 2010; Gomez et al., 2016; Obregon-Tito et al., 2015; Schnorr et al., 2014; Yatsunenko et al., 2012). Traditional foods of non-industrialized populations in Africa and South America have in several studies been found to drive the composition of the gut microbiome towards a profile particularly suited for the diet in question (De Filippo et al., 2010; Schnorr et al., 2014; Yatsunenko et al., 2012) and may be an important factor for obtaining and maintaining a diverse gut microbiome among indigenous people. As traditional lifestyles and traditional foods in non-western regions are increasingly replaced by western lifestyles and imported foods, we are at risk of losing the knowledge and skill set that has evolved around traditional foods in these regions (Kuhnlein et al., 2009). At the same time, we are potentially losing a part of the global food microbiome and alongside that a part of the human gut microbiome. Understanding the food microbiomes of traditional foods has the potential to be one way of preserving the diversity of the human gut microbiome, while also enabling a better understanding of the evolution of traditional foods.

Table 1
Drying methods used for capelin, ogac and cod.

	Drying method	Details	Drying time (d)	Drying location
Capelin	Shore – preferred taste	Collected on shore at the water-limit assumed to be slightly fermented by local expert. Preferred taste by local expert.	unknown	Qeqertarsuaq (69°N −53°W)
Capelin	Hanging in town	Hanging on house facing North	11	Ilulissat (69°N −51°W)
Capelin	Crowberry 2016	Dried on crowberry outside small settlement. Kept in freezer for one year	14[a]	Qeqertarsuaq (69°N −53°W)
Capelin	Crowberry 2017	Dried on crowberry outside small settlement. Weather was wetter and colder than previous year.	21[a]	Qeqertarsuaq (69°N −53°W)
Capelin	Industrial	Store-bought. Packaged July 2017.	unknown	Unknown
Ogac	On nets	Washed in seawater and dried on nets in the open	4	Angujaartorfik (67°N −51°W)
Cod	With gut lining	Washed in seawater and black gut lining kept on. Protected from flies.	15	Akia (64°N −51°W)
Cod	Without gut lining	Washed in seawater and black gut lining removed. Protected from flies.	15	Akia (64°N −51°W)
Cod	No sea-water	Washed in boiled and cooled freshwater. Protected from flies.	15	Akia (64°N −51°W)
Cod	Optimal	Washed in seawater and salted. Preferred method by local expert. Protected from flies.	15	Akia (64°N −51°W)
Cod	Industrial	Store-bought from same company. Two samples packaged in December 2017 and one in April 2017. Spoiled sample packaged July 2017.	unknown	unknown

[a] Approximately.

Most of our knowledge on the microbiomes of food come from fermented foods as well as industrially produced foods and is centered around pathogens rather than total microbiome and food-associated commensal microbes (Lang et al., 2014; Rizo et al., 2018). The advent of culture-independent technologies has greatly expanded our knowledge on non-industrial traditional foods (Rizo et al., 2018). The traditional Greenlandic diet is based almost solely on animal products, and is often characterized by conservation through drying (Berthelsen, 1935; Birket-Smith, 1971; Larsen and Oldenburg, 2000). Traditionally dried Greenlandic foods harbor an insofar unknown microbiome and exemplifies an underrepresented food type among food omics studies in that it is non-fermented, non-industrial and animal-sourced. The nutritional composition of traditional Inuit foods have been thoroughly mapped (Kuhnlein and Humphries, 2017), but microbiological investigations of traditional Inuit foods are rare and have so far focused on the pathogenic potential of these foods (Shaffer et al., 1990). Within the last century, the Inuit of Greenland have undergone a rapid and radical change in diet towards a "westernized" diet (Berthelsen, 1935; Helms, 1988, 1986; Pars et al., 2001). While traditional foods are still culturally important in Greenland, the increasingly rapid replacement of traditional foods with imported foods asserts an urgency in the mapping of and understanding the impact of the food microbiome of traditional Greenlandic foods. With the present study we have begun the mapping of the microbiome of traditional foods of Greenland using high-throughput culture-independent techniques.

The aim of this study was to assess whether traditional Greenlandic food-drying methods confer distinct food microbiomes and investigate the potential sources of the food microbiomes. We mapped the microbial composition on dried fish of three species from Greenland, namely capelin, cod and Greenland cod (ogac), prepared by Inuit traditional methods and industrial methods, respectively. Capelin has been an important food source for the Greenlandic Inuit, which has traditionally been dried whole and stored for winter (Berthelsen, 1935; Birket-Smith, 1971; Sinclair, 1952). To illustrate, in 1903 a Greenlandic family of four could consume around 10 kg of dried capelin in two weeks (Berthelsen, 1935). The use of capelin in fermentation and fish sauce production has been evaluated (Gildberg, 2001), but these evaluations are yet unsupported by microbiome-studies. Dried cod is one of the most popular traditional Greenlandic foods today (Pars et al., 2001) and is a prominent part of the traditional Inuit diet (Birket-Smith, 1971; Fitzhugh et al., 1977). Dried capelin and cod are both traditional Greenlandic foods that have been integrated into a modern food system and are available as industrial products on the Greenlandic market but which

are also still commonly prepared by locals according to tradition. The ogac, also part of the cod family, has traditionally been used in much the same way as the cod (Larsen and Oldenburg, 2000).

Using 16S rRNA gene amplicon sequencing we here compare the microbial composition of capelin and cod prepared with different traditional drying-methods as well as industrially produced products. Ogac samples dried in the open at a location with high presence of blow-flies were included as a test of the importance of insects as a source of the food microbiome. The analysis was focused on three hypotheses. First, we hypothesized that the capelin, which is dried including its intestinal microbiome, would have a different microbiome when compared to cod and ogac, from which only the fillet is part of the dried product. Both exterior and interior microbiomes of dried capelin were included to test this hypothesis. Secondly, we anticipate that different preparation methods would result in distinct microbiomes. Finally, we hypothesized that the environment in which the fish were dried would result in distinct food microbiomes and that insects are a potential source of the microbiome. These hypotheses were tested using clustering analysis (non-metric multidimensional scaling supplemented with ANOSIM statistics) and Indicator OTU analysis, showing which OTUs significantly represented the microbiomes on differently dried fish as well as microbiomes on different species of fish.

2. Material and methods

2.1. Sampling

All samples in this study are covered by prior informed consent from the Government of Greenland survey license number G17-025. Four methods for drying whole capelin (*Mallotus villotus*, Supplementary Fig. 1A) were tested, firstly hanging on house in town, secondly laying on crowberry, thirdly collected from the shore and finally industrially produced (Table 1). While drying on land and hanging on houses are commonly used methods, the local expert providing crowberry and shore specimens expressed a preference for the shore collected capelin as they were "tastier and better salted" (translated from *kalaallisut*). Fresh capelin skin samples were taken from live capelin straight out of the water aboard the fishing boat at Kangerluarsuk, Ilulissat, Disko Bay area, Greenland (69°N, −51°W) on the 10th of June 2017. Skin swab samples were taken using sterile cotton swabs (Aptaka, Canelli, Italy) while wearing nitrile powder-free gloves (ABENA, Aabenraa, Denmark). Edges of cotton swabs were put into 5 mL Eppendorf tubes® (Eppendorf, Hamburg, Germany) containing 3 mL RNA*later*™

(invitrogen, Vilnius, Lithuania). Capelin were brought back to land for sampling fresh capelin intestinal samples as well as drying by hanging in the town of Ilulissat (Supplementary Fig. 1C). Capelin brought back to land were transported in a bucket under environmental temperature (maximum of 9.8 °C on the date of sampling (DMI, 2019)) and were sampled within 3 h of retrieval from the ocean. Intestinal content was extracted using sterile surgical disposable scalpels (Braun, Tuttlingen, Germany). The intestine was cut out of the fish (Supplementary Fig. 1B) and the content of the distal part of the intestine was pressed into a 5 mL Eppendorf tube® containing 3 mL RNA*later*™. All capelin were laid onto a transparent plastic bag sterilized using 70% ethanol wipes before sampling. Capelin dried by hanging in town were hung less than 3 h after catching onto a house in the town of Ilulissat facing North (60°N, −51°W). Dried capelin were sampled as explained above for fresh capelin. Dried skin samples (exterior samples) were sampled by swabbing the cotton swab along the length of the dried fish. Dried interior samples were obtained by cutting up the dried fish using sterile surgical disposable scalpels (Braun, Tuttlingen, Germany), then carefully opening the fish by pulling to prevent exterior microbiome contaminating the interior microbiome, then finally swabbing the inside of the fish along its length (Supplementary Fig. 1D). Capelin dried on crowberry in 2016 and 2017 as well as capelin collected from the shore were supplied by air-mail from Qeqertarsuaq, Disko Island, Greenland (69°N, −54°W), where the fish were dried. Further details on drying methods can be found in Table 1. Samples for industrial comparisons were taken from three capelin from the same package of industrially produced dried capelin (Ammassak, Ilulissat, Greenland).

Cod (*Gadus morhua*, Supplementary Fig. 1E) fillets were dried in four different ways to test the locally preferred method (Table 1). Several locals expressed the importance of removing black gut lining from fillets before drying. One local explained that the taste will be bitter if gut lining was not removed and explained that the fillets should be washed in seawater before a slight salting. The traditional method was then compared to industrially produced winter-dried cod fillets (Royal Greenland A/S, Nuuk, Greenland). Three cod were caught for the traditionally dried samples on the 2nd of September 2017 at Akia close to Nuuk (64°N, −51°W). Fresh samples of skin were taken on the boat. Fresh intestinal samples were taken during filleting of the fish from the distal part of the intestine. Each fish was divided into 4 smaller fillets (Supplementary Fig. 1I) which were treated as described in Table 1. Fillets were dried in a drying cabin laying onto fishing-nets and behind protective nets to avoid flies. Samples for industrial comparisons were taken from three different packages from same producer of industrially produced dried cod. An additional sample was obtained from a spoiled product on which there was visible white and green mold growth (Supplementary Fig. 1J). This sample had been stored at 5 °C rather than the recommended −18 °C.

Three ogac (*Gadus ogac*, Supplementary Fig. 1H) were caught at Angujaartorfik, Kangerlussuaq fjord, Greenland (67°N, −51°W) on the 2nd of August 2017. Fresh intestines were sampled as described for capelin and cod above and dried also as described above. Ogac samples were included to test for the impact of insects on the food microbiome, since there are differing opinions locally on the importance of protecting from blowflies. The ogac samples were dried in the free with no protective nets, which resulted in visible contamination with fly excreta (Supplementary Fig. 1G). Ogac are in some regions of Greenland considered to be less tasty than cod, while in other regions it is more popular than cod (Larsen and Oldenburg, 2000). A local providing expertise on cod and ogac described dried ogac as being stronger and fishier in smell and taste than dried cod (translated from *kalaallisut*).

All drying methods described above were chosen based on what conventional drying methods are currently used in Greenland and as described by locals (personal informants, Larsen and Oldenburg, 2000). Negative controls, 5 mL Eppendorf tube® containing 3 mL RNA*later*™, were brought on sampling trips and stored and transported alongside other samples. No DNA was detected in negative samples. For all

methods, samples were taken in triplicate, from three individual fish. Some samples, however, were discarded either because of being compromised during transportation (if Eppendorf tubes® had leaked during flight) or due to unsuccessful extraction of DNA. Full list of final samples (n = 62) included in this study are listed in Supplementary Table 1.

Water activity of dried samples was measured using Decagon Pawkit Water Activity Meter (DECAGON, Pullman, WA, USA) according to manufacturer's manual.

2.2. DNA extraction and sequencing

The bacterial compositions were determined using NexteraSeq-based (Illumina, CA, USA) high throughput amplicon sequencing, targeting the V3 region of the 16S rRNA gene. Following sample delivery, samples were thawed and vortexed. Precipitation of RNA*later*™ was observed in some tubes, which were then heated and vortexed according to manufacturer's instructions (Thermo Fischer Scientific, MA, USA). DNA was isolated using the Bead-Beat Micro AX Gravity Isolation Kit (A&A Biotechnology, Poland) following the manufacturer's instructions and the samples were stored at −20 °C. DNA was handled as described previously (Krych et al., 2018) and sequenced in a single NextSeq Illumina 2x150bp run performed according to the manufacturer's instructions. Merged reads are deposited at the NIH NCBI Sequence Read Archive with accession number PRJNA548553. The presence of virulent *Shigella* was tested by PCR using primers specific for the *virA* gene, as previously described (Villalobo and Torres, 1998).

2.3. Data analysis

Read pairs were merged using Usearch version 10.0.240 *fastq_mergepairs* (Edgar et al., 2011). Merged consensus sequences were quality checked using FastQC version 0.11.5 using default settings (Patel and Jain, 2012). Usearch *fastq_filter* was used for quality filtering with minimum quality score of 25 and minimum length of 120 bases after which the sequences were quality checked with FastQC again. Quality filtered consensus sequences were pooled and the pooled data was run through the usearch pipeline for 97% identity OTU picking. In summary, unique sequences were identified with *fastx_uniques*, OTUs were clustered with *cluster_otus*, which also removes singletons and chimeras, finally the OTU table was created with *otutab* at default setting with a threshold of 97% identity. OTU table was normalized to 10,000 sequences/sample with *otutab_norm*. Before normalization the number of sequences in the samples ranged from a minimum of 196 sequences to a maximum of 110,903 sequences and an average of 47,774 sequences. Alpha diversity metrics were calculated with usearch *alpha_div* and taxonomy was assigned using usearch *sintax* with cutoff 0.7 and the RDP 16S database version 16 (Cole et al., 2014).

Beta diversity, specifically non-metric multidimensional scaling (NMDS) and Indicator OTUs (Dufrene and Legendre, 1997), was assessed in R version 3.5.0 ("R Core Team," 2018). NMDS employing Bray-Curtis distances and Indicator OTU analyses were conducted using packages vegan version 2.5-1 (Oksanen et al., 2018) and labdsv version 1.8-0 (Roberts, 2016). Indicator OTU analysis was run with 1000 iterations, p ≤ 0.5 and indicator value ≥ 0.3.

3. Results and discussion

3.1. No fish-specific difference in microbiota

When looking at all samples of dried fish, cod and ogac samples cluster together (Fig. 1, ANOSIM R = 0.114, p = 0.083) with exception of one cod sample, an optimal drying method triplicate, which is separated from all other samples. This one sample is distinct from other cod samples by being almost solely composed by *Proteobacteria* (Fig. 2). Most capelin samples cluster together next to the cod and ogac samples (ANOSIM R = 0.179, p = 0.019). Exceptions are industrial capelin

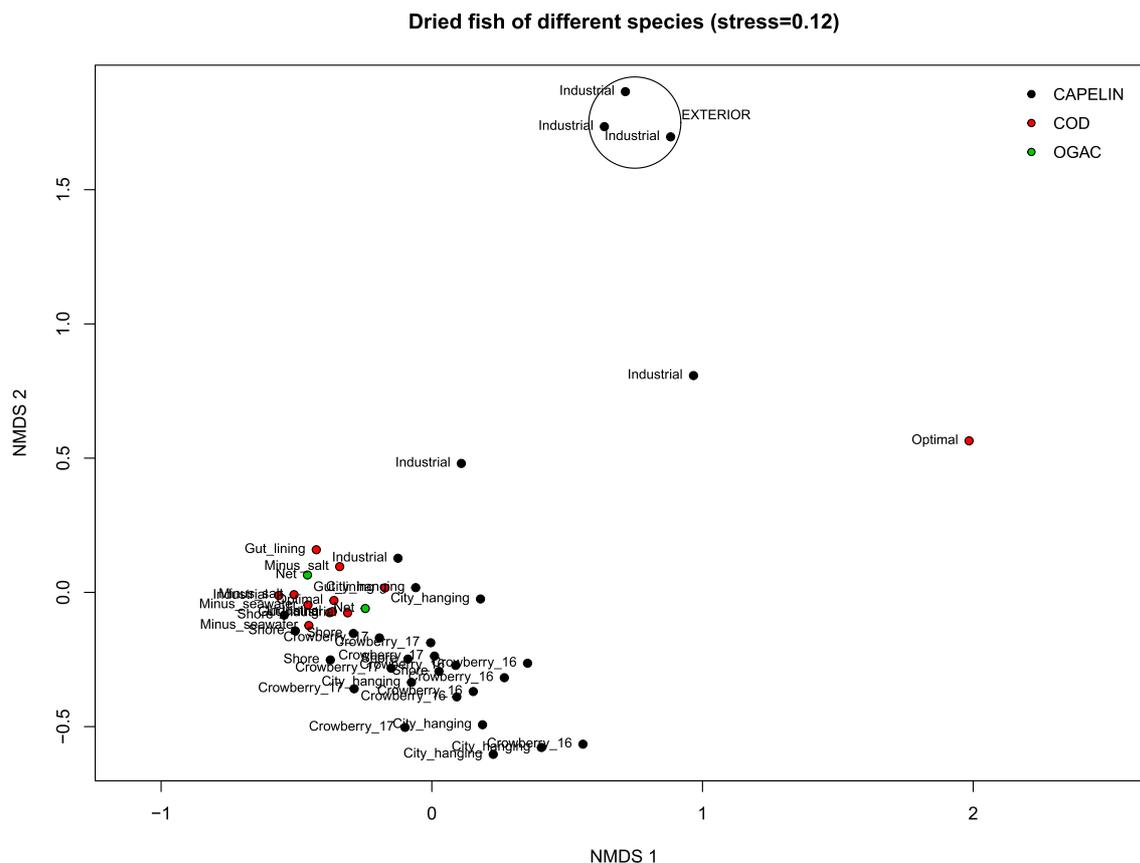**Dried fish of different species (stress=0.12)**

Fig. 1. Non-metric multidimensional scaling of dried samples from capelin, cod and ogac.

samples that separate from most other samples, the most pronounced being the exterior industrial triplicates, which form an individual cluster apart from all other samples (Fig. 1, ANOSIM R = 0.699, p = 0.001). When comparing phyla found in capelin, cod and ogac samples there are few phyla that distinguishes the three species. Exceptions are a high relative abundance of *Tenericutes* found in fresh capelin intestinal samples that are not found to be dominating in any cod or ogac samples where in turn larger fractions of *Fusobacteria* are found (Fig. 2).

There are two indicator OTUs for the capelin samples, which are significantly more abundant in the capelin samples than in the cod and ogac samples (Table 2, Supplementary Table 2). There are 29 indicator OTUs for ogac samples and none for cod. The relatively high number of indicator OTUs for ogac might be explained by the fact that there are only two ogac samples, which have been prepared in the same way at the same time. The ogac samples are therefore the most uniform group of samples, which might result in a higher number of indicator OTUs. The ogac samples were included to test whether shielding the drying fish from insects impacts the microbiome of the dried product. Ogac samples were dried in the open at a location with a high occurrence of blowflies that leave visible traces of excrement on the drying fillets (Supplementary Figs. 1F and G). Of the 29 indicator OTUs for ogac samples there are eight indicator OTUs with potential association to insects. These eight OTUs match following taxa, *Stenotrophomonas*, which has been isolated from bed bugs (Reinhardt et al., 2005). Another taxa matches *Actinomycetales* isolated from coffee berry borer among other (Ceja-Navarro et al., 2015). *Kytococcus* also isolated from *Triatoma dimidiata* (Monteon et al., 2018). *Roseomonas*, this has been isolated from whitefly (Roopa et al., 2014). Yet another taxa matches *Aerococcus* also isolated from Mexican fruit fly (Kuzina et al., 2001). Finally *Bifidobacterium* that also inhabit insect intestines such as the honey bee gut (Ellegaard et al., 2015). Four of these eight OTUs

(OTU1583, OTU1723, OTU488 and OTU1343) are not present among the cod samples, that were dried behind insect shielding nets. While these taxa may be from other sources than insects it is likely that insects are a source of the microbiome of the dried ogac and that at least some of these OTUs are contaminants from insect intestinal flora and this partly explains the high number of indicator OTUs for the two ogac samples.

We hypothesized that a potential difference in microbiome from capelin to cod and ogac would be due to the drying of the entire fish when drying capelin and thereby including the potentially abundant intestinal microbiome of the fish. The fact that capelin dried interior samples cluster more closely to cod and ogac than capelin dried exterior samples suggests that the hypothesis does not hold (Fig. 1). The indicator OTU for capelin that could be determined to genus level, *Lactococcus*, does have the gastrointestinal tract as a potential source but plants are a more common source (Jay, 1992), thus giving no support to the hypothesis. The NMDS analysis shows that there is a larger difference in microbiome within fish species than between species, suggesting that other factors than species have a greater impact on the food microbiome of dried fish when considering the three species of fish included in this study.

3.2. Capelin preparation methods result in different microbiota

For capelin, samples from different preparation methods seem to result in different microbial composition (Fig. 3A, ANOSIM R = 0.393, p = 0.001). Industrial samples separate from other methods (ANOSIM R = 0.810, p = 0.001) while distinct clustering is less prevalent among traditional samples (ANOSIM R = 0.245, p = 0.003). Industrial interior samples are spread out on the NMDS plot separated from other samples and industrial exterior samples cluster together apart from all other samples (Fig. 3A), as also discussed for Fig. 1. The observed OTU
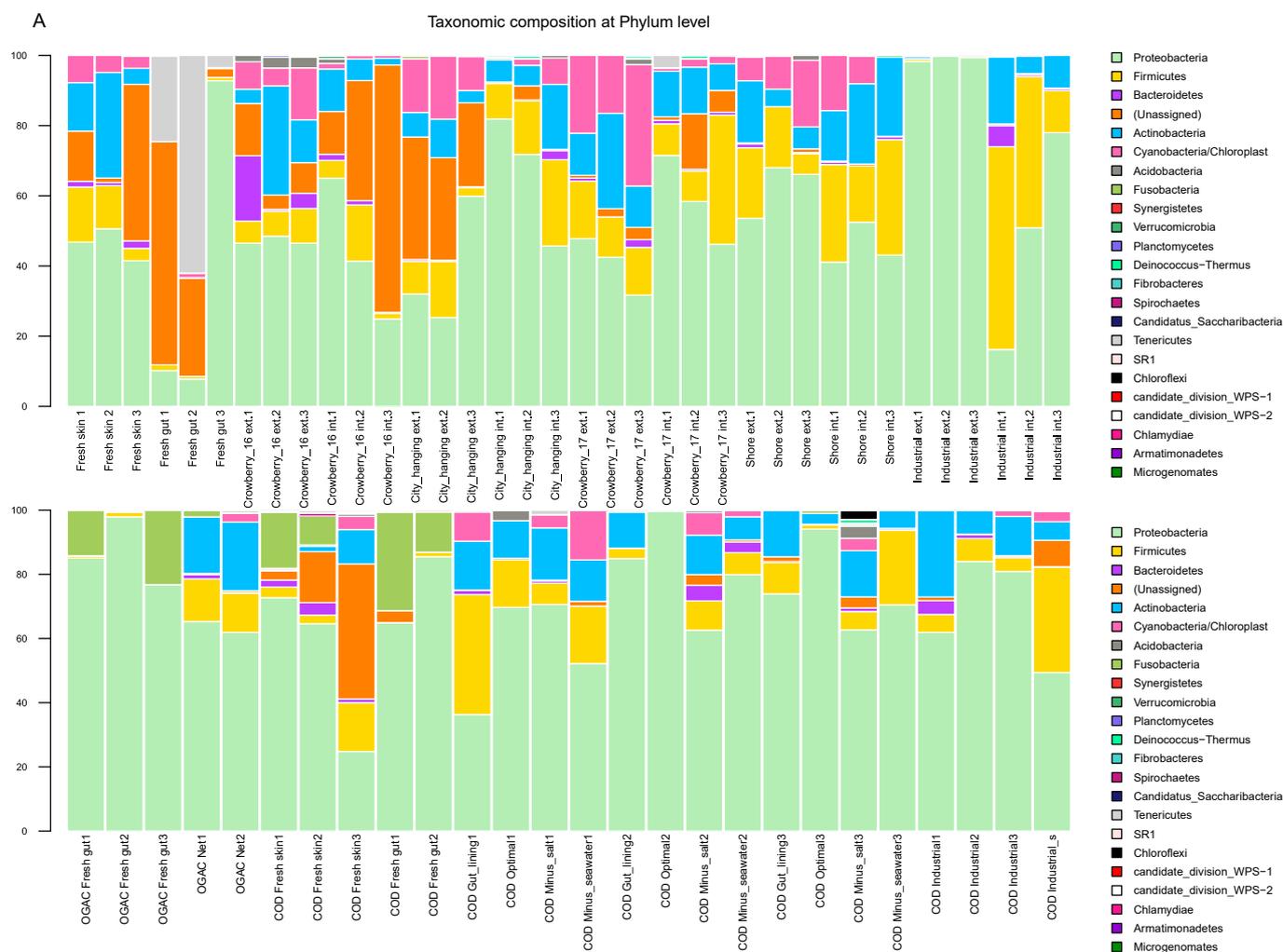
Fig. 2. Taxonomic composition of all samples at phylum level. A: capelin samples, B: ogac and cod samples.

richness observed in samples from different preparation methods range between 82 and 101 (Table 2 and Supplementary Table 1). The capelin samples have much varying fractions of unassigned OTUs at phylum level (Fig. 2). Some differences in patterns between exterior and interior samples of different drying methods can be observed, which is particularly obvious for fresh capelin samples and industrial samples (Fig. 2).

Capelin sample indicator OTUs assessment of preparation method showed that four of the five groups, i.e. town-hanging, crowberry 2016, shore and industrial, have significant indicator OTUs (Table 2). Industrial samples have the most indicator OTUs with 47 OTUs being indicator OTUs, while crowberry 2017 have no indicator OTUs. The relatively high sum of significant indicator values (42.1) suggests that there is a difference between the microbiomes of capelin dried with different methods.

A difference in the microbiome between capelin samples prepared in different settings but with no differential treatment of the fish itself, might be explained by microbial input from the differing surroundings. We would expect to find microorganisms known from the different drying-environments on the dried fish to be among the indicator OTUs if the drying-environment is a significant origin of the microbiota on the finished fish product. When looking at the taxonomic match of the indicator OTUs for samples dried on crowberry in 2016 two genera *Bryocella* (Dedysh et al., 2012) and *Granulicella* (Pankratov et al., 2007) were originally isolated from peat and one indicator OTUs matches a

land-plant chloroplast (Supplementary Table 2). Of the remaining three indicator OTUs one genus was originally isolated from a marine habitat (Biebl et al., 2007) and the last two are too broad to give any indications of origin. As the taxonomy of indicator OTUs from crowberry samples do point towards an origin from the crowberry surroundings, this gives support to our hypothesis that the environment of the samples shapes the microbiome of the finished product.

For samples from capelin collected from the shore all 9 indicator OTUs match families or genera that are known to be host-associated except one chloroplast OTU (Supplementary Table 2) such as *Propionibacterium*, *Enterobacteriaceae/Escherichia/Shigella*, *Lactococcus* and *Streptococcus*. These are all known from among other, the human gastrointestinal tract (Greenwood et al., 2007). *Lactococcus* and *Streptococcus* are homofermentative lactic acid bacteria, the latter also including potential fish pathogens (Liu et al., 2016) and finally two indicator OTUs are propionic acid bacteria. Capelin collected from the shore are different from the other samples in that the fish have not been taken directly alive from the ocean and put to dry, but have been rolling at the water line on the shore for a period before drying. The collector of the fish assumed the fish to be slightly fermented, which is supported by the presence of three lactic acid bacteria (LAB) indicator OTUs and two propionic acid bacteria (PAB) indicator OTUs. A dead fish lying on the shore will result in a prolonged time in which the fish has had a higher water activity compared to fish taken directly alive from the ocean and left to dry, thus allowing for a prolonged time in which its

Table 2
Number of Indicator OTUs (IOs) and richness (total OTU count) of groups.

Analysis	Indicator groups	Description	Number of IOs	Sum of significant IV	Mean richness
Species-indicators	Capelin	All samples of dried fish were divided into groups based on species	2		90 (sd = 37. n = 30)
	Ogac		29	17.57	79 (sd = 30. n = 2)
	Cod		0		68 (sd = 21. n = 15)
Method-indicators					
Capelin	Hanging in town	All samples of dried capelin were divided into 5 groups based on drying method	6		92 (sd = 45. n = 6)
	Crowberry 2016		7	42.1	82 (sd = 39. n = 6)
	Crowberry 2017		0		88 (sd = 43. n = 6)
	Shore		9		88 (sd = 27. n = 6)
	Industrial		47		101 (sd = 42. n = 6)
Cod	+ gut-lining	All samples of dried cod were divided into 5 groups based on drying method	2		68 (sd = 29. n = 3)
	÷ gut-lining		0		90 (sd = 22. n = 3)
	÷ seawater		1	2.6	58 (sd = 14. n = 3)
	Optimal		0		63 (sd = 11. n = 3)
	Industrial		1		60 (sd = 20. n = 3)
Industrial vs. traditional capelin	Traditional	All samples of dried capelin were divided into two groups	15	47.88	87 (sd = 37. n = 24)
	Industrial		61		101 (sd = 42. n = 6)
Industrial vs. traditional cod	Traditional	All samples of dried cod were divided into two groups	0	1.67	70 (sd = 22. n = 12)
	Industrial		2		60 (sd = 20. n = 3)

Dried cod sample from spoiled cod was not included in these assessments.
Standard deviations (*sd*) and sample count (*n*) included in brackets.

microbiome could grow in numbers. This may explain that the indicator OTUs of the capelin collected from the shore are potentially from the microbiome of the fish itself. The collector of the shore specimen expressed a preference for the taste of shore-collected capelin over crowberry dried and house-hung capelin. Lactic acid bacteria are essential for flavor formation in fermented fish (Ji et al., 2017). Propionic acid bacteria are distinguished by the production of large amounts of propionic acid by fermentation of lactic acid, a feature which is partly responsible for organoleptic properties of certain cheeses (Madigan and Martinko, 2006). The LAB and PAB indicator OTUs on shore-dried capelin potentially influences the taste of the fish, which is an interesting link between the microbiome and the local preference for certain traditional foods. Finally, the results show that both the traditional and industrial drying methods are efficient for avoiding spoilage-related fermentative microorganisms.

The 47 indicator OTUs from samples collected from industrially prepared capelin match 8 different families and genera (Supplementary Table 2). Interestingly, these are all Gammaproteobacteria, and all of them can be associated with marine or aquatic environments, many predominantly from marine or saline and cold habitats such as *Aliivibrio* (Urbanczyk et al., 2007), *Photobacterium* (Moi et al., 2017), *Moritella* (Urakawa et al., 1998), *Psychrobacter* (Juni and Heym, 1986), *Pseudoalteromonas* (Gauthier et al., 1995), *Vibrionaceae* (Madigan and Martinko, 2006) and *Psychromonas* (Mountfort et al., 1998). Samples that have been prepared by hanging in town have four broadly defined taxonomic matches for the indicator OTUs and one originally isolated from an aquatic environment (Tarhriz et al., 2013). The town environment with comparably high human activity has the potential to be more dynamic with a greater variability in sources of microbial input.

This might explain the lack of a uniform type of indicator OTUs among town-hung samples. In contrast, industrial environments are highly controlled with regard to microbial input, which may then explain the surprisingly uniform group of indicator OTUs among the industrial samples. At phylum level, industrially dried samples are all dominated by Proteobacteria, Firmicutes and Actinobacteria and some Bacteroidetes (Fig. 2). Exterior samples of industrially dried capelin are particularly uniform and are composed almost entirely of Proteobacteria (Fig. 2).

The above results on the capelin indicator OTUs for different preparation methods confirm that the environment in which the fish are dried impact the microbiome of the final product. For capelin collected on the shore our results point towards a different microbiome caused by the difference in preparation method possibly resulting in distinct taste in accordance with local knowledge.

3.3. Cod preparation does not affect microbiota

Cod samples differ from capelin samples in that these have been prepared in the same setting, except for industrial samples, but with different treatments of the fish itself. The cod samples from the 5 different preparation methods, namely with gut lining, minus salt, minus washing in sea, optimal and industrial, do not form individual clusters based on preparation method (Fig. 3B, ANOSIM R = 0.055, $p = 0.263$). An outlier (sample 52) resulted in such a large difference between this sample and the remaining samples that their ordination among each other was unreadable from the plot. Therefore, sample 52 was removed from this particular analysis. Consistent with the NMDS plot there were only four indicator OTUs among all samples and a low sum of
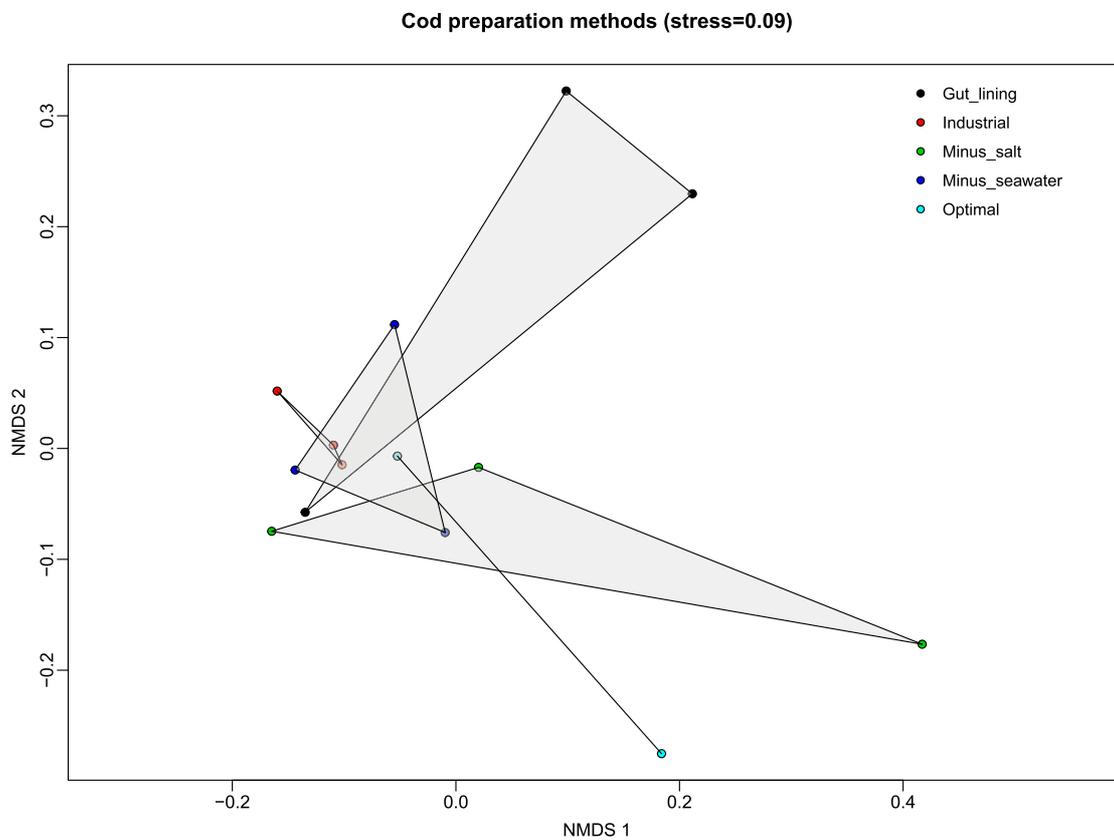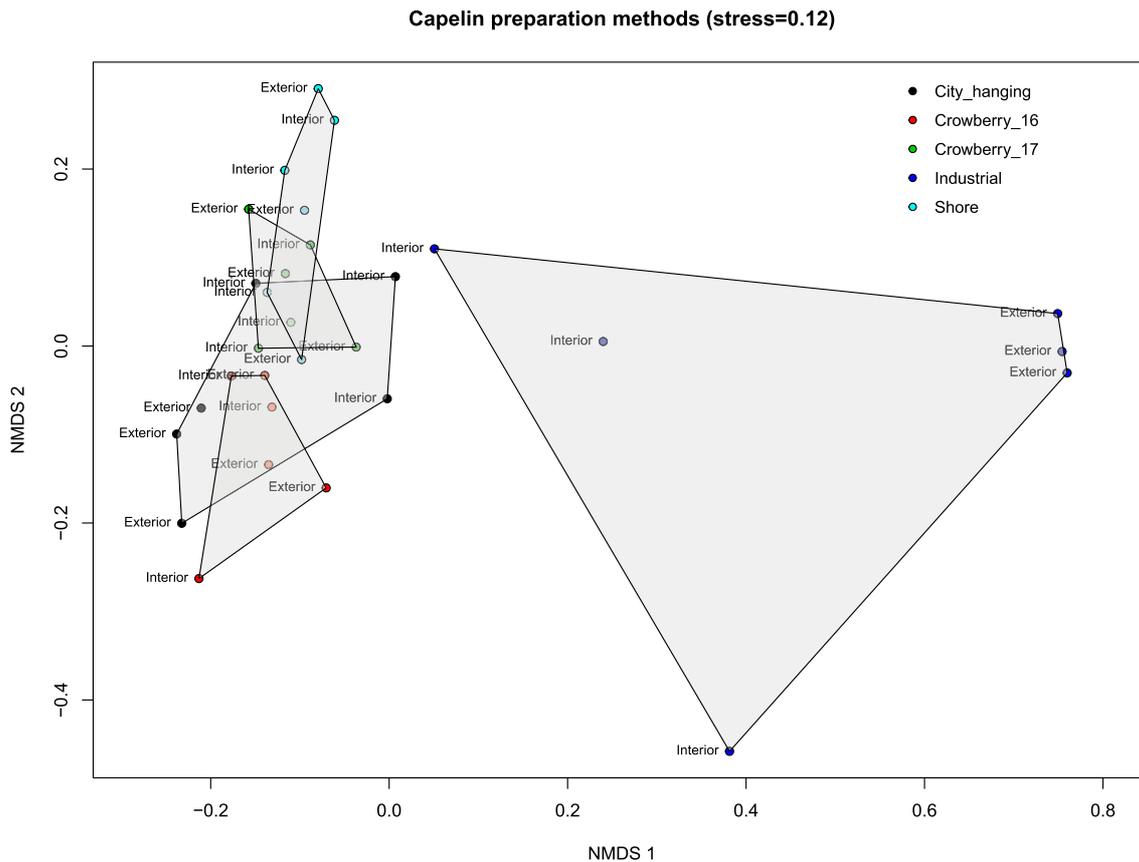
Capelin preparation methods (stress=0.12)

Cod preparation methods (stress=0.09)

Fig. 3. A: Non-metric multidimensional scaling of dried capelin samples. Convex hulls contain samples grouped by preparation method. B: Non-metric multi-dimensional scaling of dried cod samples. Convex hulls contain samples grouped by preparation method. Outlier sample 52 is not included in the analysis.

significant indicator values (Table 2).

The cod samples have a more homogenous microbiome compared to dried capelin. The traditionally dried cod samples, as opposed to traditionally dried capelin samples, were all dried in the same setting at the same time, which might partly explain the homogenous microbiome and supporting the idea, that the surrounding environment is determining the microbiome of the final product. The industrial samples, which are not from the same place and time however, also cluster among the traditional samples (Fig. 3B, ANOSIM R = 0.055, $p = 0.263$). At phylum level cod and ogac samples are dominated by Proteobacteria and Firmicutes (Fig. 2). Fresh ogac and cod samples have larger fractions of Fusobacteria, not found in to same degree in dried samples or in capelin fresh samples while dried samples of cod and ogac have larger fractions of Actinobacteria compared to fresh samples (Fig. 2).

3.4. Handling, fish-skin and ocean are sources of the microbiota

The fish intestinal microbiome is a well-known source of fish product microbiomes (Chaillou et al., 2015) which is also why fresh intestinal samples were included in present study for comparison. When comparing capelin dried exterior and interior samples to fresh skin and intestinal samples, the dried interior and exterior samples cluster closely with fresh skin samples and apart from fresh intestinal samples (Fig. 4A, ANOSIM R = 0.151, $p = 0.026$). Fresh intestinal samples separate from all other samples (ANOSIM R = 0.655, $p = 0.007$). This shows that the drying of the capelin including its intestines does not seem to be the major source of the microbiota of the dried product. For cod and ogac dried samples cluster apart from fresh samples (Fig. 4B, ANOSIM R = 0.710, $p = 0.001$). Three almost dried samples that were sampled while evaluated as not being fully done seem also to separate slightly from fully dried samples (ANOSIM R = 0.395, $p = 0.065$). The seemingly gradual separation of fresh and dry samples for cod is an interesting contrast to the lack of difference in the microbial composition of fresh skin and dried skin samples of capelin. Potentially, the additional handling of cod and ogac required for filleting the fish results in a greater deviation from the original fresh microbiota, which is absent for the capelin, that are taken almost directly from the ocean and put to dry. The lack of intestinal signal on the dried samples could in part be explained by the comparably higher diversity found on the surface skin of the fish compared to the intestines of the fish (Supplementary Table 1). For capelin, average richness for fresh skin samples was 104 observed OTUs and for intestinal samples 18 (Supplementary Table 1). For cod, fresh skin samples had an average richness of 111 observed OTUs and intestinal samples 33.5. Previous studies have shown that environmental bacteria on fish are more resistant to processing than the intestinal microbes from fish (Chaillou et al., 2015). Additionally, it can be speculated that more opportunistic organisms will pass through the skin microbiome than the more protected intestinal microbiome of the fish, resulting in a higher potential of the skin microbiome to survive on dried meat.

The genera *Blautia* (Liu et al., 2008) and *Cetobacterium* found among the most abundant OTUs are well known from the intestinal flora, the latter with a specific niche in fish intestines (Urakawa et al., 1998) (Supplementary Table 3). The present study confirms that intestinal microbes from fish are also found on traditionally processed fish but shows that the skin microbiome is a more important source of the dominant microbes on the dried products in accordance with previous studies (Chaillou et al., 2015). These results support above conclusions that the dominant microbes in the microbiomes of dried fish is derived from the surrounding environment.

Other than the fish intestinal and skin microbiome an expected source of microbes on the dried product is the ocean environment, which is not only the origin of the product but is also used traditionally to wash the fresh fillets before drying. Among the 20 most abundant OTUs across all samples are *Erythrobacter* (Zheng et al., 2016), *Aliivibrio*

(Urbanczyk et al., 2007), *Photobacterium* (Moi et al., 2017), *Psychrobacter* (Maruyama et al., 2000) and *Croceicoccus* (Xu et al., 2009), which are taxa that contain well-known marine species. Again, this supplements above results that the environment plays a crucial role as input to the microbiome on dried fish.

3.5. Impact of industrial versus non-industrial drying of fish

The microbiome of industrially produced capelin differs from those of traditionally prepared capelin (Fig. 3A). Samples of industrially produced capelin have 61 indicator OTUs when compared to the combined group of all the traditionally prepared capelin (Table 2). The comparably large number of indicator OTUs and concurrent higher sum of significant indicator values is not surprising considering the close clustering of the industrial samples apart from traditional samples in the NMDS analysis (Fig. 3A, Table 2).

For traditionally prepared capelin there are three Bacilli out of 15 indicator OTUs while industrially prepared capelin have the anaerobic counterpart represented with five out of 61 indicator OTUs being clostridia. This might indicate that the industrial process of drying selects for anaerobic organisms. However, there are no other strict anaerobes among the remaining indicator OTUs for industrially dried capelin and several aerobic indicator OTUs (Supplementary Table 2).

There are 12 different identified genera and families within four phyla among the 15 indicator OTUs for traditionally dried capelin (Supplementary Table 2). For industrially dried capelin there are only 9 different identified genera and families among the 61 indicator OTUs of which are all either Gammaproteobacteria or Clostridiales with one exception being an Actinobacteria (Supplementary Table 2). These results show a more homogenous group of indicator OTUs for industrially dried capelin when compared to traditionally dried capelin, as also discussed in section 3.2. The indicator OTU assessment is supported by the taxonomic composition of the total OTUs, which also shows a more homogenous microbial composition of industrial samples (Fig. 2). This might be explained by a more controlled and streamlined environment in which the industrially dried capelin are produced. Interestingly, the potentially more controlled industrial samples have few unassigned OTUs at phylum level, while fresh samples and the traditional samples crowberry 2016 and city-hanging have larger fractions of OTUs that cannot be assigned to phylum level (Fig. 2). This underlines that the study of traditional food microbiomes is a resource for discovering yet undescribed food-associated microbes that potentially affect our intestinal microbiome.

When comparing industrially dried cod with traditionally dried cod there are two indicator OTUs for industrially dried cod (Table 2 and Supplementary Table 2). For cod, industrial samples clustered closely and among samples from other drying methods (Fig. 3B). Results from the indicator OTU analysis is in accordance with these results in that there was a comparably lower sum of significant indicator values and only two indicator OTUs for industrially dried cod and none for traditionally dried cod (Table 2).

In summary, the comparison of industrial and non-industrial samples shows a different pattern for capelin than for cod. For capelin, industrial production results in a different microbiome than traditional production. For cod, there seem to be little difference between traditionally dried cod and industrially dried cod, despite the fact that dried cod from the industry were dried at a different location and at a different season.

3.6. Food safety

Only one sample was considered spoiled at the time of sampling. This spoiled industrial cod sample was not included in above analysis, but had a comparably higher richness than other industrial samples and included eight OTUs that were not present in any other sample (Supplementary Table 1). Among the eight OTUs that are unique to the
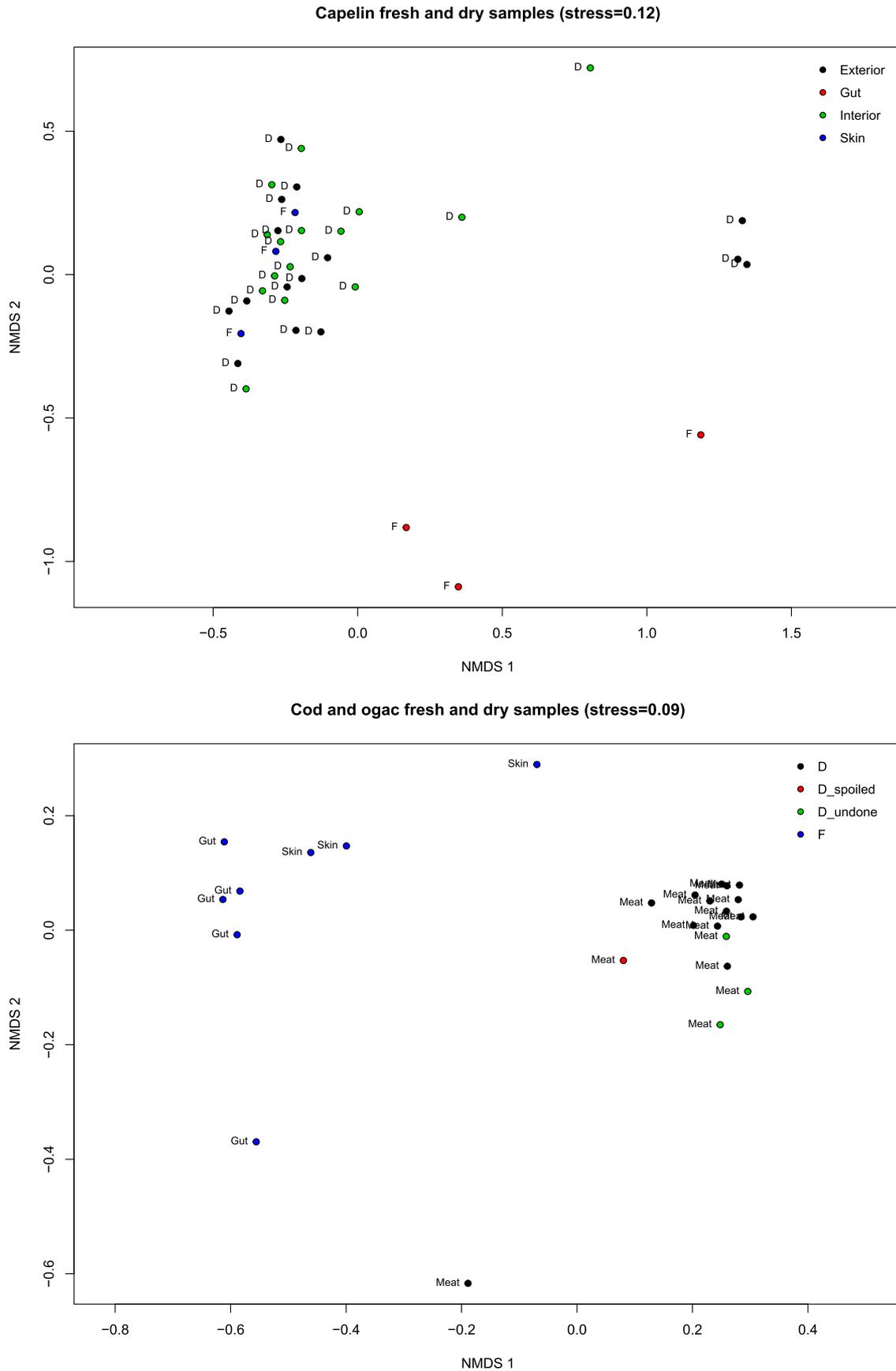
Capelin fresh and dry samples (stress=0.12)

Cod and ogac fresh and dry samples (stress=0.09)

Fig. 4. A: Non-metric multidimensional scaling of all capelin samples grouped by drying stage and inner/outer samples, i.e. skin (outer, fresh), gut (inner, fresh), exterior (outer, dry), interior (inner, dry). B: Non-metric multidimensional scaling of all cod and ogac samples grouped by drying stage. D = dry, D_spoiled = a spoiled dry sample, D_undone = a dried sample that was evaluated as not yet done at sampling time, F = fresh.

spoiled sample are three OTUs with taxonomies that can be related to factory environments, including *Hyphomicrobium* known from water and soil but also represented in sewage treatment plants (Holm et al., 1996), *Saccharibacteria*, isolated from among other activated sludge and water treatment plants (Hugenholtz et al., 2001) and *Anoxybacillus* isolated from among other a milk processing plant (Goh et al., 2014). This suggests that microbes originating from the industrial processing of fish are part of the spoilage microbiome of dried fish.

The most abundant OTU (OTU1737, Supplementary Table 3) when all samples are considered together matches *Escherichia/Shigella*. As organisms of the genus *Shigella* are well-known causes of food-borne illness (Jay, 1992), further assessment of the presence of virulent *Shigella* was conducted using *virA* PCR amplification. No *virA* product was detected using PCR amplification targeting the *virA* gene in any of the samples.

4. Conclusions

In conclusion, environmental and processing conditions appear more important than fish species in determining the microbiome on the dried fish. For capelin, different preparation methods confer different microbial compositions. Taxonomic analyses indicated that the more controlled industrial environment results in a more uniform microbiota. Intriguingly, for capelin collected on the shore our results point towards a different microbiota caused by the difference in preparation method possibly resulting in distinct taste which is in accordance with local knowledge. The present study also confirms that intestinal microbes from fish are found on traditionally processed fish but shows that the skin microbiome is a more important source of the dominant microbes on the dried products. Furthermore, the results showed that the marine environment is also a source of the microbiome. The present study serves as a beginning of the mapping of the traditional Greenlandic food microbiome and as an initial reference point for future studies into the evolution of traditional foods in Greenland.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.103305.

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