

Effects of different cooling techniques on bacterial succession and other spoilage indicators during storage of whole, gutted haddock (*Melanogrammus aeglefinus*)

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Abstract Effective cooling of newly caught fish is of great importance to inhibit bacterial growth and therefore increase quality, safety and shelf life of the product. In this study, two commercial cooling media (liquid ice A and B) were tested and their performance was compared to conventional plate ice during chilled 8-day storage of whole, gutted haddock. Temperature was monitored, and deteriorative changes were followed by conventional microbiological counts [(total viable psychrotrophic; specific spoilage organisms and physicochemical methods (pH, TVB-N, TMA, salt content)]. A cultivation-independent method (16S rRNA clone analysis) was used to study the effect of cooling treatments on the bacterial community of haddock initially and at the end of storage. The results show that the bacterial growth behaviour observed for differently cooled fish was not supported by their temperature profiles. Growth of the SSOs, *Photobacterium phosphoreum* and H₂S-producing bacteria was delayed at early storage, independently of the cooling methods. With further storage, little or no count differences were seen among traditionally iced fish and those cooled in liquid ice with a top ice layer. At the end of storage, significant ($p < 0.05$) increase in *P. phosphoreum* and H₂S-producing bacteria counts of skin and flesh sampled from liquid ice with no top ice layer was observed along with higher salt, TVB-N and TMA flesh content. Cultivation-independent analysis confirmed the dominance of *P. phosphoreum* in fish stored

in liquid ice B with no top layer (up to 76% dominance) and liquid ice A with top layer (44% dominance). *Psychrobacter* and *Flavobacterium* dominated the microbiota of fish stored in conventional plate ice and liquid ice B with ice top layer. The study shows that the use of liquid ice prepared from brine provides faster initial cooling of whole fish but may create unfavourable conditions under extended storage where the active spoiler *P. phosphoreum* becomes dominant. Plate ice may therefore be an optimal medium for extended fish storage.

Keywords Cooling · Haddock · Bacterial succession · Specific spoilage organisms · *Photobacterium phosphoreum* · 16S rRNA gene

Introduction

Effective cooling prolongs the shelf life of food and fish in particular. Different attempts have been approached to delay the spoilage of fresh fish, using superchilling techniques [1], modified atmosphere packaging [2], a combination of both [3, 4] or even radiation [5]. Changes of environmental conditions will delay or even stop the typical spoilers from growing, but at the same time, a new niche is opened, allowing the onset of other bacteria. Because of the short shelf life of fish, traceability and information on quality parameters are of great importance during trading of fish. It has been shown before that the correlation between the quality as judged by sensory evaluation and total bacterial counts is not as accurate when compared to the levels of specific spoilage organisms (SSO) [6, 7]. Knowledge on how the microbiota will respond to different cooling methods can help to better understand the spoilage process and design cooling

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methods that can prolong shelf life, as well as to improve bacterial growth models and pinpoint the most relevant biosensors for evaluation of fish freshness.

Cooling technologies have been improving in recent years with the development and commercialisation of slurry ice machines of different kinds where various mixtures of water, ice, salt and gases have been used to increase cooling rate and optimise temperature during transportation. All these developments aim at reducing bacterial growth and thereby extending the shelf life and increasing the quality of the product [8]. Several different slurry ice production technologies and a number of slurry ice brands exist (e.g., Liquid Ice, Flow Ice, Optim-Ice, Fluid Ice and Bubble Slurry Ice). The thermal and rheological properties along with other characteristics of ice slurries have been thoroughly described [9]. Information on how these new techniques affect the succession of the microbiota present on the skin and flesh of the fish is limited at present.

Examples of well-documented fish spoilage organism are members of the *Pseudomonas* genus and *Shewanella putrefaciens* which are frequently used as bacterial indicators for spoilage and are present in different food types [10–12]. *Photobacterium phosphoreum* has not been investigated as intensively but there are scientific evidences that it is the main spoilage organism when environmental conditions are favourable such as in chilled modified atmosphere packed fish [4, 13, 14]. Other workers have reported its presence in aerobically spoiled fish [15, 16] and its importance in the spoilage of air-stored gadoid fish products was recently reported [1, 17].

Since the emergence of molecular biology and PCR-based strategies, a completely new vision on microbial diversity has been established. These methodologies have shown the presence of uncultivable populations and lateral gene transfer between species which has greatly complicated the picture for microbiological evolution and speciation of bacteria. These new insights are in large proportions from the ocean microbiota which is likely to influence the bacterial composition on any fish species living in the same surroundings [18, 19].

The aim of the present study was to investigate the practical application of four different cooling methods available to the fish industry for whole fish storage with regard to their cooling performance and effects on fish deterioration using conventional methods such as microbial enumeration by plate counting and physicochemical analysis. Furthermore, the treatments' effect on bacterial community changes during storage was evaluated using a cultivation-independent method (16S rRNA clone analysis).

Materials and methods

Experimental setup

Four cooling treatments were evaluated during the 8-day storage trial of whole haddock in October 2008: (1) Plate ice (PI) with a top layer of plate ice (PI + PI) constituted 100 kg of fish layered with 45 kg of plate ice (with a top layer of ice) in a drained tub (310 L); (2) liquid ice A with a top layer of ice (LIA + PI) prepared with 100 kg of fish layered in 100 kg of liquid ice A (ice ratio = 34%); (3) liquid ice B with no top layer of ice (LIB), with 100 kg of fish layered in 100 kg of liquid ice (ice ratio 47%); (4) LIB + PI prepared as for LIA + PI but using liquid ice B. The tubs in treatments 2, 3 and 4 were chilled for 2 h, undrained and then drained followed by top icing (12 kg PI) for treatments 2 and 4. Haddock was caught in Hvalfjordur using a longline, gutted and rinsed upon landing and was received at the laboratory almost un-iced 12-h post-catch.

Temperature logging

In each of the four tubs comprising five layers of fish, temperature loggers were inserted into the fish flesh in four individuals weighing approximately 600–1,300 g, positioned at the bottom, middle and top layers, resulting in 12 loggers recording the fish flesh temperature every 5 min for each treatment. These loggers were iButton type DS1922L (Maxim Integrated Products, Sunnyvale, CA, USA) with an accuracy of ± 0.5 °C, a resolution of 0.0625 °C and an operating range of -40 – 85 °C. In addition to those loggers, one temperature logger was used to measure ambient temperature outside each tub and another one to record the medium temperature, approximately 10 cm below surface. These were Onset temperature loggers, type UTBI-001 (Onset Computer Corporation, Pocasset, MA, USA), with an accuracy of ± 0.2 °C, a resolution of 0.02 °C and an operating range of -20 – 70 °C.

Sampling of whole, gutted fish

At initial sampling (day 0), four fish were removed aseptically from the fish tubs, pooling two fish per sample. During subsequent sampling (d2–4–8), four fish were removed aseptically from the top layer of the differently treated tubs, pooling two fish per sample for each treatment examined. Fish were transferred to the laboratory in expanded polystyrene foam (EPS) boxes and analysed within 30–60 min.

Cultivation-dependent microbial analyses

Microbiological counts were evaluated for both the skin and the flesh of haddock. Two pieces of skin were aseptically cut from one side of each fish (two fish = one sample), giving a total area of 30 cm², and mixed with 60 mL of cooled Maximum Recovery Diluent (MRD, Oxoid, Hampshire, UK) for 1 min in a stomacher (Stomacher Lab Blender 400, A.-J. Seward Laboratories, London, UK). Successive tenfold dilutions were done as required. The other side of each fish was aseptically skinned, pieces of flesh removed, minced and diluted tenfold in cooled MRD. Cultivation methods used are described by Olafsdottir et al. [1]. Presumptive *Pseudomonas* were cultivated on modified Cephaloridine Fucidin Cetrinide (CFC, 22 °C) agar (Oxoid) based on Stanbridge and Board [20], H₂S-producing bacteria on Iron Agar (IA, 17 °C), *Photobacterium phosphoreum* with Malthus conductance method [21] and total viable psychrotrophic counts (TVC) on Long and Hammer's medium (LH, 17 °C). All samples were analysed in duplicate and results presented as an average. Microbiological quality of freshly prepared ice media (PI, LIA, LIB) was evaluated on LH, IA and CFC following serial dilution of melted samples.

Chemical analyses

The minced flesh samples prepared for microbiological analyses were used. The method of Malle and Tao was used for total volatile bases (TVB-N) and trimethylamine (TMA) measurements. TVB-N was measured by steam distillation (STRUERS, Copenhagen, Denmark) and titration, after extracting the fish mince with 7.5% aqueous trichloroacetic acid solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulphuric acid solution. TMA was measured in trichloroacetic acid (TCA) extract by adding 20 mL of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine. All chemical analyses were done in duplicate.

The pH measurements were performed in 5 g of mince moistened with 5 mL of deionised water. Salt (NaCl) content was measured with the Volhard Titrimetric method according to AOAC ed. 17 from 2000 (no. 976.18).

DNA extraction for cultivation-independent analysis

Upon sampling, tenfold diluted fish samples were collected and kept at –20 °C until DNA extraction. Prior to extraction, the duplicate samples of each treatment were pooled and 1 mL (0.1 g) of this preparation was used for DNA extraction. Raw material (day 0), four skin samples

(day 8) and four flesh samples (day 8) were selected for 16S rRNA clone analysis. Template genomic DNA was isolated as described before [22].

Cultivation-independent bacterial analysis

The 16S rRNA analysis was performed in order to determine the dominating bacterial groups in early and late storage and to obtain a separate measure on bacterial succession than cultivation alone. PCR reaction was done by amplifying the 16S rRNA gene with universal primers, 9F and 805R (5'-GAGTTTGATCCTGGCTCAG-3' and 5'-GACTACCAGGGTATCTAATCC-3', respectively). PCR conditions, cloning and sequencing of PCR products obtained from the fish samples were performed essentially as described before [23]. From each pooled sample, a total of 48 clones were picked for sequencing resulting in 384 sequencing reactions in total. The results were analysed with Sequencer 4.8, built 3767 (Gene Codes Corporation, Ann Arbor, MI, USA) and identified with BLAST search through NCBI 16S rRNA database.

Statistical analysis

Statistical analysis of microbial and chemical data was carried out with NCSS 2000 software (UT, USA) using analysis of variance methods (Kruskal–Wallis one-way ANOVA on ranks for salt content and one-way ANOVA for all other variables). Comparison of data with respect to treatments was performed using the Kruskal–Wallis multiple comparison z -value test and Fisher's LSD multiple comparison test, respectively. The threshold for significance was 0.05.

Results

Temperature monitoring

The treatments including liquid ice B provided slightly faster cooling than the other two media tested, but they did not maintain the fish temperature as well throughout the 8-day storage period. In those LIB groups, 0 °C was reached in 21–28 min, but 57 min with liquid ice A. When plate ice was used solely, it took 262 min to reach 0 °C (Fig. 1). The average fish temperature in the different cooling treatments ranged from –0.09 to –0.64 °C (Table 1). The temperature in the PI + PI and LIA + PI increased by approximately 0.2 °C during the storage period and by 0.8 and 0.9 °C with LIB and LIB + PI, respectively (Fig. 1). No significant difference was found in the mean ambient temperature between groups (data not shown). During the 8-day storage, the temperature fluctuated between 2 and

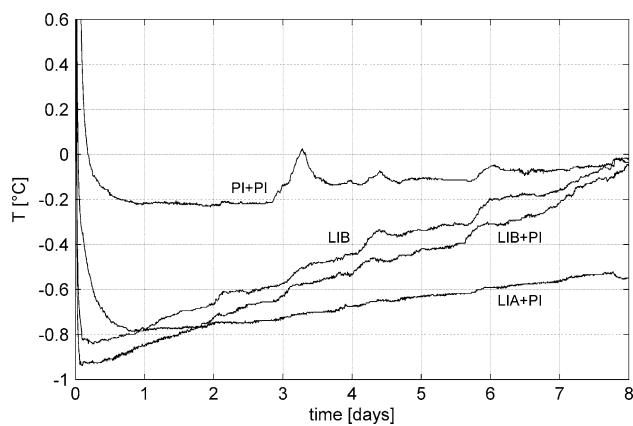


Fig. 1 Maintenance of temperature—mean temperature of all layers (initial fish temperature = 7.5 °C) over the 8-day storage period in the differently treated fish. Samples were stored in plate ice with a top ice layer (PI + PI), liquid ice A with a top ice layer (LIA + PI), liquid ice B with (LIB + PI) or without (LIB) a top ice layer

5 °C for all the groups with a mean temperature of 3.4 ± 0.8 °C.

Effects of cooling treatments on physicochemical changes in haddock flesh

Measurements of pH in haddock flesh throughout the storage time showed little variation and were constantly in the range of pH 6.5–6.7 (data not shown). Initial NaCl content of fish flesh was found to be $0.15 \pm 0.07\%$, while the salt content of the cooling media ranged from 0 to 2.9% (Table 1). After 2 and 4 days of storage, a slight salt uptake was noticed, but after 8 days, a significant ($p < 0.05$) increase was found in all liquid ice treatments compared to the NaCl content of the raw material (Fig. 2). Initial TVB-N content in the raw material amounted to 11.9 ± 2.8 mg N/100 g flesh. After 8 days of storage, a significant ($p < 0.05$) increase in TVB-N and TMA was found for liquid ice treatments compared to traditional icing (PI), reaching the highest levels when an ice top layer was not applied (LIB) (Fig. 2).

Microbial changes during storage using cultivation-dependent methods

Analysis of freshly prepared ice media indicated the higher microbial contamination of LIB and plate ice compared to LIA (Table 1). Skin counts were assessed initially on the raw material used, resulting in an overall psychrotrophic load (TVC) of $\log 3.1 \pm 0.1$ colony-forming units (cfu)/cm². *P. phosphoreum* (Pp, $\log 2.9 \pm 0.3/\text{cm}^2$) was found to dominate among the SSO assessed (H_2S -producers, $\log 2.2 \pm 0.3$ cfu/cm²; pseudomonads, $\log 0.8 \pm 0.3$ cfu/cm²). The growth behaviour of these SSO was influenced by the cooling treatments, as shown in Fig. 3. The least effect was observed during the first 4 days of storage for pseudomonads which grew steadily, independently of the cooling methods used. However, after 8 days of storage, pseudomonad counts were significantly ($p < 0.05$) lower on fish cooled with LIB + PI than LIB alone or LIA + PI. Ice storage performed as well as LIB + PI in slowing down pseudomonads. Pp growth was delayed for at least 4 days by all cooling methods, after which a significant growth increase was seen on fish stored in LIB compared to other treatments ($p < 0.05$). A similar but shorter delay was seen for H_2S producers, followed by significant growth in LIB-cooled fish. Overall, the psychrotrophic skin microbiota developed significantly faster on LIB-stored fish than in other treatments.

Since the flesh of a newly caught fish is generally known to be sterile, the flesh at day 0 was not analysed. Psychrotrophic flesh counts were slightly influenced by the cooling treatments on day 2, but no significant differences were seen for TVC and pseudomonad counts among the four treatments as time progressed (Fig. 4). *P. phosphoreum* growth in fish flesh was delayed by all cooling treatments for at least 4 days, coinciding with the results for skin counts (Figs. 3, 4). After 8 days of storage, slowest Pp growth was seen in LIB + PI and PI + PI stored fish but fastest growth was observed in LIB-stored fish with no ice on top. At that time point, H_2S producers had reached

Table 1 Measured parameters of each cooling treatment during the storage experiment

Group	Average fish temperature °C (±SD)	Salt content (%)	TVC ^a Log ₁₀ cfu/mL	H ₂ S ^a Log ₁₀ cfu/mL	Pseud. ^a Log ₁₀ cfu/mL
PI + PI	-0.09 (±0.31)	0	4.6	2.9	2.4
LIA + PI	-0.64 (±0.24)	2.9	2.6	<1	<1
LIB	-0.39 (±0.33)	2.1	5.8	3.0	3.5
LIB + PI	-0.47 (±0.34)	2.1	5.8	3.0	3.5

Fish stored in plate ice with a top ice layer (PI + PI), liquid ice A with a top ice layer (LIA + PI), liquid ice B with (LIB + PI) or without (LIB) a top ice layer

^a Microbial counts (TVC, total viable psychrotrophic counts; H_2S -producing bacteria and *Pseudomonas* counts) in the different ice media (PI, LIA or LIB) freshly prepared

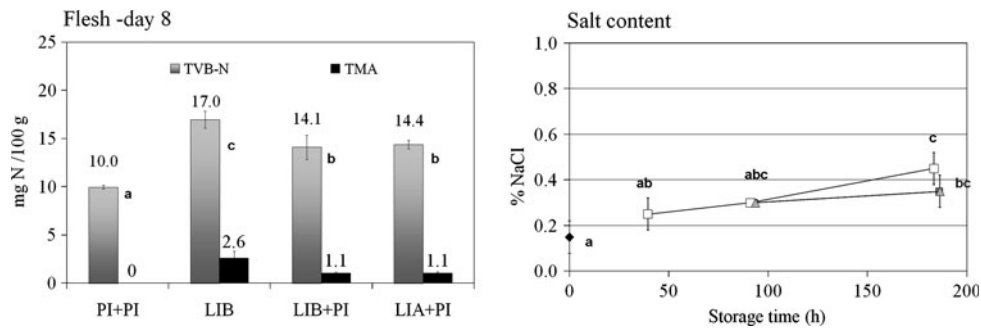
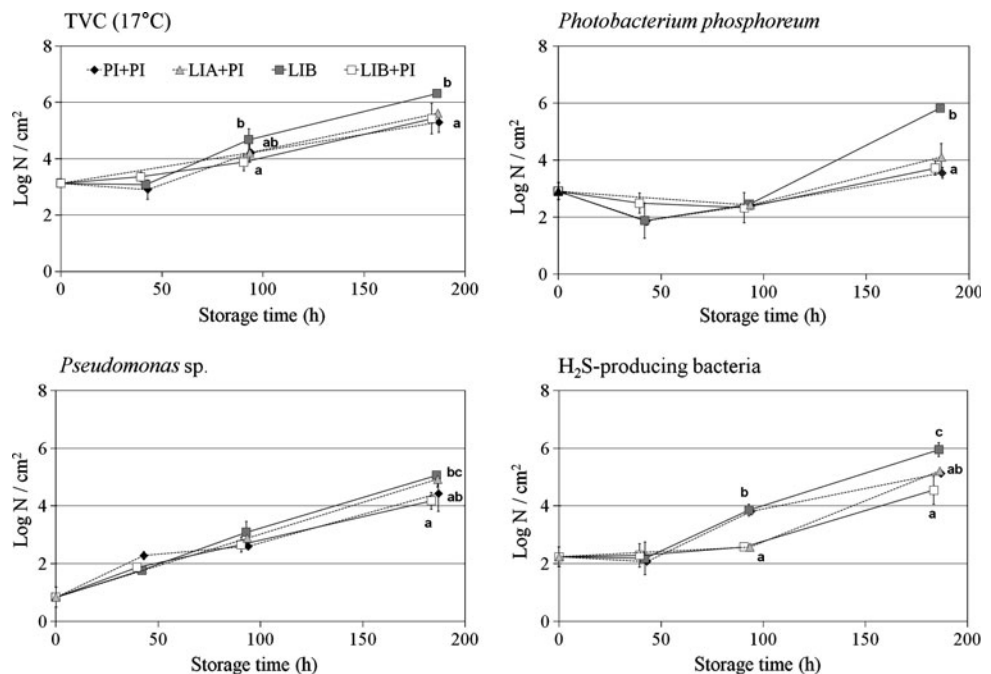


Fig. 2 Production of volatile basic compounds and P ratio (% NaCl) in haddock flesh after the 8-day storage period as influenced by the cooling treatments. Fish stored in plate ice with a top ice layer

(PI + PI), liquid ice A with a top ice layer (LIA + PI), liquid ice B with (LIB + PI) or without (LIB) a top ice layer. Different letters indicate significant differences among cooling treatments ($p < 0.05$)

Fig. 3 Development of microbiota on haddock skin as influenced by the cooling treatments. Fish stored in plate ice with a top ice layer (PI + PI), liquid ice A with a top ice layer (LIA + PI), liquid ice B with (LIB + PI) or without (LIB) a top ice layer. Different letters indicate significant count differences among cooling treatments on respective sampling day ($p < 0.05$)



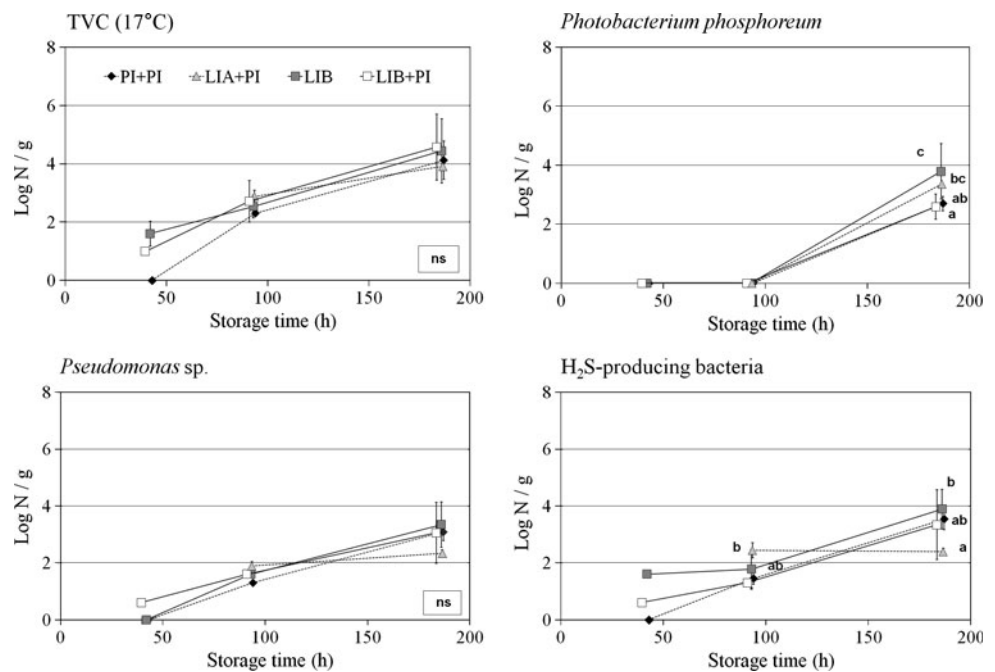
lowest levels (log 2.4 cfu/g) in LIA + PI stored fish, but highest (log 3.3–3.9 cfu/g) in the other groups (Fig. 4).

Bacterial community changes during storage using a cultivation-independent method

From all the samples analysed, a total of 64 different species were detected in 348 successful sequencing reactions (Table 2). The skin bacteria of newly caught, gutted and rinsed haddock showed a highly diverse population with 26 species and 15 genera with no single dominating species (Fig. 5). *Vibrio*, *Acidobacteria* and *Mycoplasma* sp. showed somewhat higher relative abundance than others. The largest group is a collection of sequences that corresponded to uncultured species and could therefore not be identified. After 8 days of storage in different cooling treatments, large shifts in bacterial dominance were

observed with reduced diversity when compared to the skin sample on day 0. Generally, a good agreement was observed between the skin and flesh microbiota composition for each treatment. Storage in plate ice (PI + PI) resulted in the lowest bacterial diversity compared to the other groups where *Psychrobacter* was the dominating species along with *Flavobacterium* to a lesser extent. The proportions of these bacteria were inverted in the LIB + PI group, where *Flavobacterium* became dominant at the expense of *Psychrobacter*, while the total bacterial numbers remained similar between the groups (Figs. 2, 5). A different scenario was observed in both LIA + PI and LIB groups where *Photobacterium* was most apparent but several other genera were also detected (Fig. 5). The LIB group, however, contained highest number of bacteria according to the plate count enumeration and highest relative abundance of *P. phosphoreum* (Fig. 2). No results

Fig. 4 Development of microbiota in haddock flesh as influenced by the cooling treatments. Fish stored in plate ice with a top ice layer (*PI + PI*), liquid ice A with a top ice layer (*LIA + PI*), liquid ice B with (*LIB + PI*) or without (*LIB*) a top ice layer. Different letters indicate significant count differences among cooling treatments on respective sampling day ($p < 0.05$); *ns* not significant



are presented for the skin sample from the LIA + PI group since PCR amplification was not successful.

Discussion

The present study shows the effects of different cooling media on selected physicochemical and microbiological parameters of whole, gutted haddock over an 8-day period. It reveals the sensitivity and dynamics of bacterial populations that develop in fish during storage at subzero temperatures and how minor changes in storage conditions can impact the microbiota in this environment.

The results show that liquid ice provides faster cooling than plate ice alone. However, a top layer of plate ice was found necessary to maintain better the superchilled condition established in liquid ice treatments during extended storage. The average fish temperature in the LIB + PI tub raised more rapidly ($0.11\text{ }^{\circ}\text{C day}^{-1}$) between days 1 and 8 compared to the LIA + PI treatment ($0.04\text{ }^{\circ}\text{C day}^{-1}$) which could be explained by the larger ice crystals of LIA than LIB, hence maintaining better cooling and delaying the melting process. Despite the temperature difference observed between plate ice and liquid ice treatments, no significant difference was observed in skin microbial load among these treatments after 2 days of storage. However, significantly faster SSO growth was observed on LIB fish after 4 and 8 days of storage, coinciding with the ongoing average temperature increase seen in the fish. After 8 days, no or little difference was seen among the other treatments.

As evidenced in our study, SSO growth was not only temperature dependent, but was apparently influenced by the environmental conditions established in fish stored using liquid ice treatments. On day 8, the salt content had doubled to tripled in LI-stored fish compared to iced fish and may explain the more rapid development of *P. phosphoreum* under these conditions. This increased salt uptake might be explained by the 2-h undrained cooling period during initial storage of LI treatments, causing salt deposition on the fish skin. Sodium chloride lowers the freezing point of flesh waterphase, hence protecting bacteria to some extent, and because *P. phosphoreum* is a sodium-requiring bacterium [10], it can be expected that increasing salt concentration in haddock flesh stimulated its growth. A recent study, reporting on the effect of a 2-day brining immersion treatment of cod fillets prior to air-packaging, showed that shelf life of superchilled brined products was lesser than that of unbrined fish, explained by slightly faster SSO growth and enhanced production of basic volatile compounds [4, 24]. Indeed, TVB-N and TMA increase in flesh of LIB fish was highest, coinciding with the SSO proliferation described. No increase in TVB-N was observed in iced fish after 8 days of storage, in agreement with the undetected level of TMA, indicating a much slower spoilage process in conventionally iced haddock.

The impact of the cooling treatments on the quality preservation of whole, gutted haddock, as studied by the evaluation of microbial and chemical spoilage indicators, was well reflected by the evolving skin and flesh bacteria in the differently treated fish. The 16S rRNA clone analysis

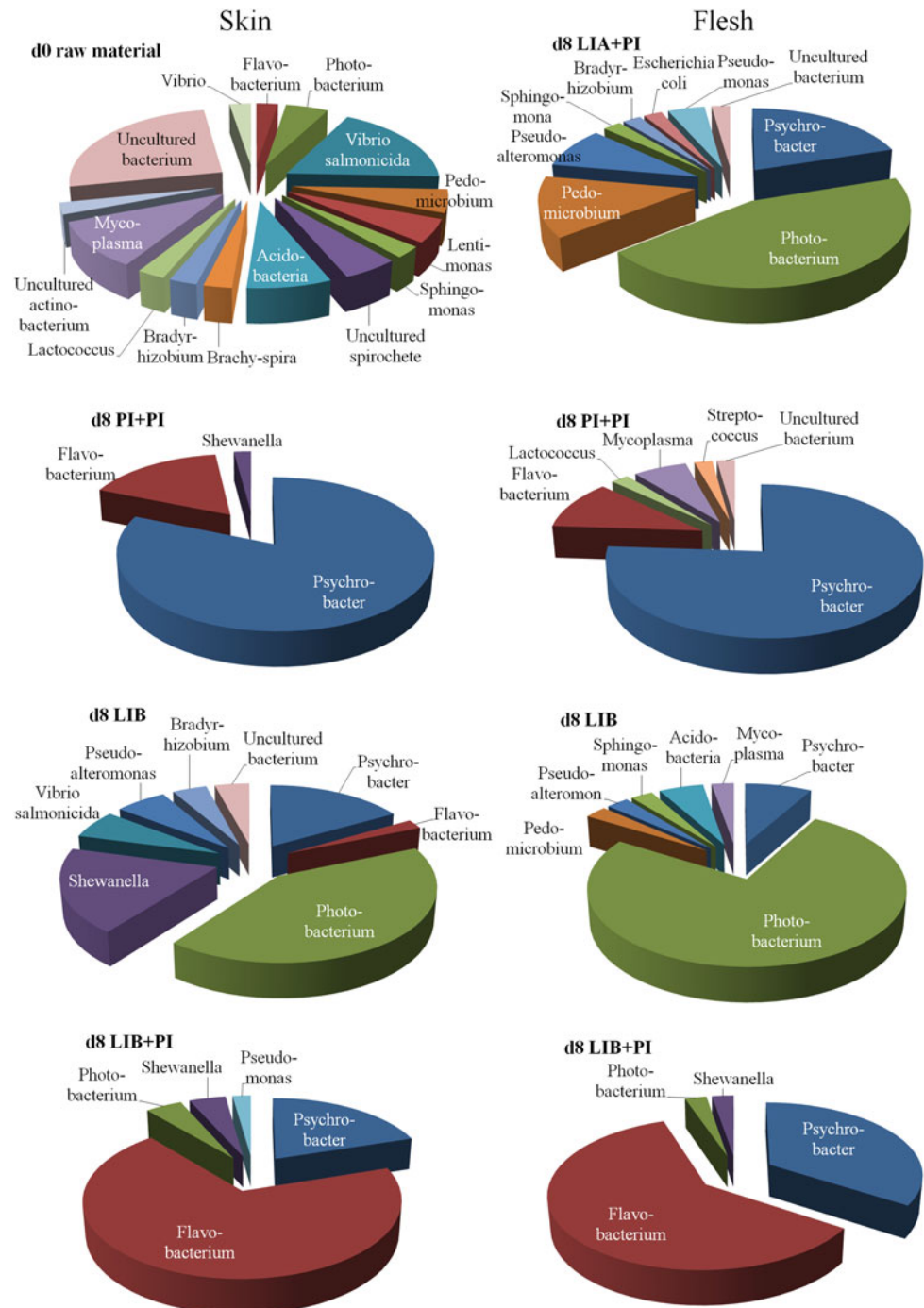
Table 2 Predominant species identified in the pooled haddock samples by 16S rRNA gene analysis

Closest NCBI database match	Accession number	Identity (%)	Number of clones	Incidence (%)
<i>Psychrobacter</i> spp.	AF260715	99	95	27.3
Uncultured <i>Flavobacterium</i>	AY494683	98	61	17.5
Uncultured <i>Photobacterium</i> spp.	AM183731	100	59	17.0
<i>Psychrobacter maritimus</i>	EU000245	100	11	3.2
Uncultured <i>Photobacterium</i> spp.	AM183746	98	11	3.2
<i>Vibrio salmonicida</i>	EU091324	99	10	2.9
<i>Shewanella</i> spp.	FJ626841	99	10	2.9
<i>Pedomicrobium australicum</i>	X97693	98	9	2.6
<i>Mycoplasma wenyonii</i>	EF221880	99	7	2.0
<i>Psychrobacter aquimaris</i>	AY722804	98	4	1.1
Uncultured <i>gamma proteobacterium</i>	AM259792	91	4	1.1
<i>Flavobacterium frigidarium</i>	AB455257	99	3	0.9
Uncultured <i>Bradyrhizobium</i> spp.	DQ303345	99	3	0.9
<i>Pseudomonas</i> spp.	AF331664	100	2	0.6
Uncultured <i>spirochete</i>	DQ340184	94	2	0.6
Uncultured <i>bacterium clone A309</i>	FJ456836	98	2	0.6
<i>Sphingomonas rhizogenes</i>	AY962684	96	2	0.6
Uncultured <i>Acinetobacter</i> spp.	AY588958	99	2	0.6
Uncultured <i>bacterium clone</i>	EU765114	94	2	0.6
<i>Lentimonas marisflavi</i>	EF157839	96	2	0.6
<i>Flavobacterium</i> spp.	U85888	99	2	0.6
<i>Pseudoalteromonas</i> spp.	FJ461453	99	2	0.6
<i>Acinetobacter calcoaceticus</i>	FJ263920	99	2	0.6
Additional species detected as a single clone	Various		41	11.8
Total number of clones analysed			348	100
Total number of species detected			64	

showed considerable differences in the developed bacterial population after 8-day storage among the different cooling treatments. The liquid ice treatments maintained some of the heterogeneity of the skin bacteria, dominating in *Photobacterium*, *Shewanella*, *Psychrobacter* and *Flavobacterium* among others, while iced fish (PI + PI) microbiota was restricted to the three latter genera. *Photobacterium* was not recovered from the skin of iced fish. Generally, the transition of skin bacteria into the flesh of iced fish was mostly proportional where microbial composition of both skin and flesh was comparable. At end of storage, the PI + PI fish flesh was dominated by *Psychrobacter* (76%) and *Flavobacter* (11%) to a lesser extent. These proportions were interestingly inverted in the LIB + PI group where *Flavobacter* showed 61% dominance and *Psychrobacter* 34%. The bacterial flora of LIA + PI and LIB was dominated by *Photobacterium* (45 and 75%, respectively) which is in agreement with cultivation and TMA formation. Whether these differences in bacterial community structure are strictly the consequence of different ice properties or contamination of ice

media cannot be ascertained. The ice media used in the present study had different bacterial loads (log 2.6–5.8 cfu/mL) which can clearly affect microbial developments and reduce shelf life. Previous investigations have shown the presence of *Salinibacter* in high dominance in liquid ice as well as incidence of different bacteria such as *Acinetobacter*, *Psychrobacter*, *Arcobacter* and *Flavobacter* in other samples (unpublished results). In our study, the different ice media were prepared from the same water source, so chemical analysis of the media was restricted to their salt content, being the most influential factor for bacterial growth behaviour in the fish. The capability of liquid ice to rapidly lower fish temperature to a subzero temperature justifies its use for fish cooling. The ultimate aim is to reduce bacterial growth as much as possible to delay fish deterioration and therefore the salubrity of the ice medium is essential to minimise risks of bacterial contamination. It has been shown that SSO growth in air-stored gadoid fish fillets is influenced by temperature, especially *P. phosphoreum* being enhanced under temperature abuse but delayed at superchilled conditions (−1 °C).

Fig. 5 Relative distribution of bacterial populations in whole, gutted haddock sampled as a raw material prior to storage and after 8 days of storage in tubs using different cooling media. Skin bacteria (d0 for initial sample and d8 for 3 treatments) is shown to the left and flesh bacteria (d8) to the right for each treatment. Fish stored in plate ice with a top ice layer (PI + PI), liquid ice A with a top ice layer (LIA + PI), liquid ice B with (LIB + PI) or without (LIB) a top ice layer



H_2S -producing bacteria behave similarly but to a lesser extent, while pseudomonads are less influenced [1, 17]. Other studies have shown the superior performance of slurry ice storage (-1 to -1.5 °C) when compared to ice alone (0 – 1 °C) during storage of various fish species resulting in lower microbial loads, higher score in sensory analysis and shelf life extension at superchilled conditions [25–27]. Furthermore, the use of ozonated slurry ice increased the storage performance of farmed turbot and sardine even further [28, 29]. Salt uptake was not evaluated

in these studies and could not be detected by the sensory method used, but was reported during slurry ice storage of horse mackerel [30]. The bacterial developments occurring in fish stored in slurry ice are likely to be species specific where gadoid and other marine fishes containing TMAO might be more susceptible for *P. phosphoreum* growth and related deteriorative changes. In the present study, it is suggested that the more salty and wet environment created by the liquid ice may have contributed to the transition of *P. phosphoreum* from the skin to the flesh and influenced

its development despite this superchilled condition, which is supported both by cultivation-dependent and cultivation-independent analysis. It has been shown by another research group that ozonated slurry ice did not extend the shelf life of MA-packed ozonated cod fillets due to prevailing levels of *P. phosphoreum* [31].

In conclusion, this study has shown the effects of different commercial cooling media on quality parameters on whole, gutted haddock. Furthermore, the influence of the cooling media on the bacterial species composition was monitored and showed a significant divergence of bacterial developments explained by the environmental conditions caused by the cooling treatments applied to the fish. The use of liquid ice is in many cases the most suitable cooling medium for whole fish to achieve faster cooling. One drawback of liquid ice is its stimulating growth effect on *P. phosphoreum* during extended storage of marine cold-water fish as a consequence of increased salt concentrations although draining the tubs during initial cooling might minimise this effect.

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