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Microbiological assessment along the fish production chain of the Norwegian pelagic fisheries sector – Results from a spot sampling programme

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ABSTRACT

Microbes play an important role in the degradation of fish products, thus better knowledge of the microbiological conditions throughout the fish production chain may help to optimise product quality and resource utilisation. This paper presents the results of a ten-year spot sampling programme (2005–2014) of the commercially most important pelagic fish species harvested in Norway. Fish-, surface-, and storage water samples were collected from fishing vessels and processing factories. Totally 1 181 samples were assessed with respect to microbiological quality, hygiene and food safety. We introduce a quality and safety assessment scheme for fresh pelagic fish recommending limits for heterotrophic plate counts (HPC), thermos tolerant coliforms, enterococci and *Listeria monocytogenes*. According to the scheme, in 25 of 41 samplings, sub-optimal conditions were found with respect to quality, whereas in 21 and 9 samplings, samples were not in compliance concerning hygiene and food safety, respectively. The present study has revealed that the quality of pelagic fish can be optimised by improving the hygiene conditions at some critical points at an early phase of the production chain. Thus, the proposed assessment scheme may provide a useful tool for the industry to optimise quality and maintain consumer safety of pelagic fishery products.

1. Introduction

Fisheries around the world provide food and income, along with traditional cultural identity. Worldwide, the annual fish catches landed in 2006–2012 were stable at around 90 million metric tonnes, with the largest volumes originating from marine fisheries. During this period, the Peruvian anchoveta (*Engraulis ringens*) was caught in highest volumes, followed by Alaska pollock (*Theragra chalcogramma*), skipjack tuna (*Katsuwonus pelamis*), Atlantic herring (*Clupea harengus*), and chub mackerel (*Scomber japonicus*) (Food and Agriculture Organization, 2014). Some pelagic fish species comprise the largest proportion of the marine catches since large volumes are still used in animal feed production, *i.e.* not directly intended for human consumption (Tacon and Metian, 2009). According to the Directorate of Fisheries, the pelagic fisheries sector in Norway comprises more than 100 ocean-going vessels, and about 150 fish processing operators. The catching volume accounted for half of the total Norwegian wild catch fisheries, exceeding 1.2 million tonnes in 2014. This gave a first-hand value of 4.9 billion NOK, with Atlantic mackerel (*Scomber scombrus*), herring (*C. harengus*), blue whiting (*Micromesistius poutassou*) and capelin (*Mallotus villosus*) accounting for around 90%. Herring contributes with one third of both catch volume and value, whereas Atlantic mackerel is the most valuable pelagic species per weight of freshly landed fish (Directorate of Fisheries (2014)). Approximately 85% of Atlantic mackerel and herring landed in Norway are bound for export, with Russia, Denmark, China, and Japan as the main markets (Norwegian Seafood Council, 2014). The global demand for high quality food resources is expected to increase steeply, and since most wild captured fish stocks are already fully exploited, or even over-exploited, a further increase in demand for fishery products must be based on better and more efficient utilisation of the already harvested resources. Globally, as much as 25% of the fish are wasted post-harvest (Food and Agriculture Organization, 2015), and the responsibility to optimise the utilisation of the resources lies to a large extent within the fish industry.

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Along the chain, from capture to landing and processing, the fish is in contact with surfaces of handling equipment, as well as storage and washing water within the production environment. During this contact, contamination by microorganisms may occur through the water, personnel and inadequate cleaning procedures. Most pelagic fish are round-frozen at the factories, bound for export. Proper temperature control is essential during storage, transportation, and production, to minimize bacterial growth prior to freezing. Additionally, fish carry an indigenous microbiota that includes the specific spoilage bacteria (SSB) of fish (Svanevik and Lunestad, 2011) and in a few cases, potential human pathogens *e.g. Listeria monocytogenes* and *Clostridium botulinum* (Huss, 1997). The muscle tissue of healthy fish is assumed sterile upon catch, whereas bacteria are typically found on all outer surfaces (skin, shell, and gills) as well as in the alimentary tract. Still, newly harvested fish from cold waters are usually considered to represent a low risk with respect to consumer hazards (Feldhusen, 2000; Painter et al., 2013), where scombrotoxin (*i.e.* histamine) intoxication is most frequently reported (Huss et al., 2000). Either way, the fish sector has to comply with challenges along the production line, including adequate cleaning- and disinfection routines (Regulation (EC) No 853/2004). Several studies have been performed to identify biohazards, and to analyse and calculate the risk of contamination of different food items during processing (den Aantrekker et al., 2003; Kusumaningrum et al., 2003; Pérez-Rodríguez et al., 2008), including marine species *e.g.* Atlantic salmon and Atlantic herring (Bagge-Ravn et al., 2003; Skåra et al., 2011). A Hazard Analysis and Critical Control Points (HACCP) plan is, however, not required for fishing vessels, as they are defined as primary producers. In addition to general requirements regarding production of safe food, the vessels are obliged, by regulation, to use clean tools and clean water (FOR-2008-12-22-1623).

Heterotrophic plate counts are important indicators of fish quality and cleanliness at various contact points on board of fishing vessels and in processing factories. The presence of hydrogen sulphide (H_2S) producing bacteria indicates the remaining shelf life, as the proportion of H_2S-producing bacteria normally is low immediately post capture, but increases during storage and processing. As most food borne pathogens are transmitted through the faecal-oral route, strict rules concerning hygiene apply to all food producers. To assess the hygienic conditions in the production environments, analyses for specific indicator organisms of faecal contamination would be needed. In the present study, the coliforms, thermo-tolerant coliforms, and presumptive *Escherichia coli*, as well as enterococci, were in focus. To assess the food safety, the examination of different pathogenic species are required. *L. monocytogenes* occurs naturally in marine environments influenced by run off from land, and could therefore follow the fish throughout the production line. Other bacteria, such as *Staphylococcus aureus* and *Salmonella*, are more often associated with cross-contamination during production. Thus, as a human pathogen, *Salmonella* is of much greater concern for seafood in the southern parts of Europe and in the US (Amagliani et al., 2012), as members of this group are rarely found in fish products in Norway, but sometimes in fish feed and at the fish feed factories (Lunestad et al., 2007). Nevertheless, analyses are included to ensure the absence of these bacteria for the export market.

In this paper we introduce an assessment scheme to evaluate the microbiological conditions of fresh fish, surfaces and production water along a production line. The scheme was applied on the results from ten years of spot sampling in the Norwegian pelagic fish sector. Samples were assessed for quality, hygiene, and food safety, aiming to improve and further optimize quality of fish products during production.

2. Materials and methods

2.1. Location and species

The present study focused on Atlantic mackerel (*S. scombrus*), two herring stocks (*C. harengus*, North Sea- and Norwegian spring spawning (NSS) herring), blue whiting (*M. poutassou*), and Barents Sea capelin (*M. villosus*). The study followed the catch through the entire production line under authentic commercial conditions. This included purse seiners and trawlers of the Norwegian ocean-going fishing fleet, all equipped with laboratory facilities, and various fish processing factories. Forty-one (41) samplings were carried out, where seven fishing vessels (Vessel A-G) were involved in 29 different samplings, including mackerel (15), North Sea herring (6), NSS herring (3), blue whiting (4), and capelin (1). Additionally, six different factories (Factory A-F) were examined at 12 different samplings which included mackerel (10) and NSS herring (2). Samples of fish and contact points (surfaces and water) were collected from all vessels and factories, however, not necessarily on every cruise. One exception was Vessel E, where no water samples were taken. All samplings are listed in Table 1. It should be noted that, given the large number of vessels and factories involved in the Norwegian pelagic fisheries, our samplings reflect only a small fraction of the annual production volume. However, the Norwegian pelagic industry is characterised by comparatively young vessels (most built in 2005 or later) and modern processing factories which use basically the same technology (and supplier). Thus, our samples reflect most likely an average microbiological situation within the sector.

2.2. Samples

During the sampling period, 628 fish were sampled and analysed at both commercial fishing vessels (453) and fish processing factories (175). Fish samples were aseptically collected by hand, with glows washed with 70% ethanol, and put in sterile sampling bags prior to storage on ice for transportation to the laboratory. On board the vessels, fish were collected from the refrigerated seawater (RSW) tanks prior to landing, whereas at the fish processing factories, fish were collected from the landing tanks, as well as at different critical stages throughout the production line.

Samples collected from the surfaces of equipment and water associated with fishing and processing are collectively referred to as contact point samples (*i.e.* water and surfaces in contact with the fish). From vessels, surface samples were collected from the pump nozzle, sift box, sorting chamber, RSW storage tank, tubes, and outlets, primarily before capture. At some samplings, we examined the purse seine or trawl bag. Seawater samples were taken during on-board pumping, and from the RSW tanks prior and after storage of fish, and kept in sterile 500 ml bottles. At the factories, surface samples included conveyor belts, sorting and filleting machines, in addition to surfaces of water drains in the production area. Water samples were taken from the landing tanks, either seawater or tap water, and different washing tanks inside the factory holding potable water. Some samples were collected from the clothing of the workers that were in contact with the fish. Overall, 533 contact point samples were examined.

2.3. Heterotrophic plate count and H_2S-producing bacteria

The heterotrophic plate count (HPC) of fish samples were examined by cultivation using Iron Agar Lyngby (Oxoid), which also gives the number of H_2S-producing bacteria as black colonies, due to precipitation of iron sulphide (FeS) (Gram, 1992; Gram et al., 1987). Preparation was done according to the Nordic Committee

Table 1
Overview of all samplings from fishing vessels and fish processing factories, the examined species, and the number (n) of examined fish-, surface-, and water samples at each sampling.

Sampling#	Location	Species	Fish (n)	Surface (n)	Water (n)	Comments
1	Vessel A	Atlantic mackerel	10	12	10	To Factory D (#2)
2	Factory D	Atlantic mackerel	10	11	6	From Vessel A (#1)
3	Vessel B	Atlantic mackerel	5	17	14	To Factory B (#4)
4	Factory B	Atlantic mackerel	8	18	6	From Vessel B (#3)
5	Vessel C	Atlantic mackerel	11	19	12	To Factory F (#6)
6	Factory F	Atlantic mackerel	8	9	4	From Vessel C (#5)
7	Vessel D	Atlantic mackerel		6	4	
8	Vessel A	Atlantic mackerel	4	12	4	To Factory F (#9)
9	Factory F	Atlantic mackerel	14	9	4	From Vessel A (#8)
10	Vessel B	Atlantic mackerel	8	12	4	To Factory C (#11)
11	Factory C	Atlantic mackerel	9	14	4	From Vessel B (#10)
12	Vessel C	Atlantic mackerel	8	12	4	To Factory F (#13)
13	Factory F	Atlantic mackerel	9	11	6	From Vessel C (#12)
14	Vessel D	Atlantic mackerel	8	12	4	To Factory B (#15)
15	Factory B	Atlantic mackerel	9	13	6	From Vessel D (#14)
16	Vessel D	North Sea herring	8	12		
17	Vessel D	Blue whiting	9	1		
18	Vessel C	Atlantic mackerel	8	12	4	To Factory F (#19)
19	Factory F	Atlantic mackerel	9	7	4	From Vessel C (#18)
20	Vessel B	Atlantic mackerel	4	6	3	To Factory E (#21)
21	Factory E	Atlantic mackerel	12	10	2	From Vessel B (#20)
22	Vessel D	Blue whiting	23	31	3	
23	Factory A	Atlantic mackerel	16	11	2	
24	Factory E	NSS herring	44	14	2	
25	Vessel G	NSS herring	50			
26	Vessel D	Capelin	10	7		
27	Vessel D	Blue whiting	7	9	2	
28	Vessel G	North Sea herring	50			
29	Factory E	NSS herring	27	4		
30	Vessel G	Blue whiting	20			
31	Vessel G	North Sea herring	10	18	5	
32	Vessel F	Atlantic Mackerel	20		8	
33	Vessel F	NSS herring	20	14	10	
34	Vessel F	Atlantic mackerel	20	14		
35	Vessel E	Atlantic mackerel	20			
36	Vessel G	North Sea herring	20			
37	Vessel E	North Sea herring	20	13		
38	Vessel E	Atlantic mackerel	20	6		
39	Vessel E	Atlantic mackerel	20	6		
40	Vessel E	North Sea herring	20	7		
41	Vessel E	NSS herring	20	7		
Total			628	396	137	

on Food Analysis (NMKL) method 184 (NMKL, 2006). Black colonies and all colonies were enumerated and the results were reported separately as log CFU/g.

The HPC examination of surfaces was performed either by contact plates with non-selective agar or by dip-slides (Hygicult) for Total Plate Count (TPC). All samples were incubated aerobically at 30 °C, for 48 h and all results were reported as log CFU per cm^2, after comparing samples to the supplied results scheme from the producer. Water samples were examined by embedding 1 ml and 0.1 ml water into Water Plate Count Agar (WPCA) before aerobic incubation at 30 °C, for 72 h. Results were reported as log CFU/ml.

2.4. Indicator organisms for faecal contamination

To reveal possible faecal contamination, the presence of indicator organisms was examined. Analysis for enterococci was performed according to the NMKL method 68 (NMKL, 2011). Results were reported as log CFU/g, and the detection limit were 2.0 log CFU/g.

Analyses for coliforms, thermo tolerant coliforms, and presumptive *E. coli* were performed from a homogenate of 10 g muscle tissue with skin in peptone water. During the ten years of this study, three different methods were applied including the NMKL method 125 (NMKL, 2005), the Most Probable Number (MPN) NMKL method No. 96 (NMKL, 2009b), and by a Petrifilm™ (3M™ Coliform Count Plates) method, according to the protocol supplied by the producer. All results were reported as log CFU/g and the detection limit were 1.0 log CFU/g.

Samples from surfaces, were tested for the presence of bacteria in the Enterobacteriaceae family, either by contact plates with Violet Red Bile Glucose agar (VRBG) or by dip-slides (Hygicult) for Enterobacteriaceae (one side) and β-glucuronidase positives (one side) *i.e.* presumptive *E. coli*. These samples were incubated aerobically at 37 °C, and the colonies were counted after 24 and 48 h. The colony density was reported as CFU per cm^2 after comparing samples to the supplied results scheme (Hygicult).

From water samples, 100 ml were filtered through a 0.45 μm membrane filter, and the filter was transferred to appropriate agar plates. For enterococci, m-Enterococcus agar was incubated at 44 °C for 48 h, whereas m-Endo agar LES (Difco) was used for coliforms, and incubated at 37 °C for 24 h. For thermo-tolerant coliforms, m–FC– agar (Difco) was incubated at 44.5 °C for 24 h. Blue colonies on m–FC– agar were confirmed as *E. coli* by inoculation and incubation in EC-broth, prior to indole testing.

2.5. Coagulase positive staphylococci

Fish samples were analysed for coagulase positive staphylococci

were examined by the NMKL method 66 (NMKL, 2009a). Results were reported as log CFU/g and the detection limit was 1.0 log CFU/g. Contact point samples were not tested for staphylococci.

2.6. L. monocytogenes

Two different methods were used for the detection of *L. monocytogenes*. Firstly, we combined NMKL method 136 (NMKL, 2010) and miniVIDAS (BioMérieux), whereas the other used the chromogenic agar RAPID'L.mono (Bio-Rad), performed according to the protocol supplied by the producer. Both methods were based on 25 g of muscle tissue with skin. Supplementary samples, using the same swab on the skin of 10 individual fish, were counted as one sample. Analyses were performed similar as for tissue samples, and the results reported as either positive or negative.

Contact point samples were collected from surfaces, either by dry swabs or pre-moist swabs, swabbing an area of 25 cm^2. Samples from drains were collected by leaving two tampons in each drain for 1 h. The analysis was performed by the same protocol used for fish samples described above. Results were reported as negative or positive. Some surfaces were analysed by Path-Check Hygiene Swabs (Microgen Bioproducts), where any positive samples were confirmed in the laboratory similar as for fish samples. To analyse the water samples, 100 ml were filtered through 0.45 μm membrane filters, before the filter were transfer to a stomacher bag, and analysed as done for fish and swab samples.

2.7. Salmonella

Fish were examined for the presence of *Salmonella* by two different methods. One method combined the cultivation as described in the NMKL method No. 71 (NMKL, 1999) and confirmation by the Enzyme Linked Fluorescent Assay (ELFA) applied in the miniVIDAS (bioMérieux) system. The other method was based on the chromogenic agar RAPID' Salmonella (Bio-Rad) according to the protocol supplied by the producer. Both methods were run on 25 g samples of muscle tissue with skin. Results were reported as positive or negative samples. Swab sampling was performed as for *L. monocytogenes*, and the swabs were analysed according to the methods described for fish. In some cases, the Path-Check Hygiene Swabs (Microgen Bioproducts), were used.

2.8. Computing and graphs

The results were treated in Microsoft Excel 2013, where all bacterial counts (X) were log transformed (log (X + 1)). To test for differences, statistical testing of HPC and H_2S-producing bacteria of fish samples collected from the vessels, the landing tank and the fish processing factories. Gaussian distribution by D'Agostino-Pearson omnibus normality test and One-Way ANOVA Tukey's multiple comparisons test, were performed in GraphPad Prism 6.0 (GraphPad Prism Software, Inc.), where also graphs were prepared.

2.9. Evaluation of the results

To evaluate the results, an assessment scheme was outlined from the contemporary EU regulations and amendments, including the regulation on hygiene of foodstuff, and the microbiological criteria for foodstuffs (Regulation (EC) No 853/2004; Regulation (EC) No 2073/2005). However, no guidelines for acceptable conditions specific for fish are set for any microorganism other than *L. monocytogenes*, which should not be found in any fish product, or in the production environment. For the evaluation of the other parameters, where currently no EU regulations applies, the former Norwegian quality regulations for fish and fish products (FOR-1996-06-14-667) and the food hygiene regulations (FOR-2008-12-22-1623) were included, as these were the acting laws at the time of many samplings. Since all kinds of water that is involved in food production should hold potable quality, the Council Directive on the quality of water intended for human consumption (Council Directive 98/83/EC) also applies. For assessment of the cleanliness of surfaces, the evaluation form provided from the Hygicult protocol (Orion Diagnostica Oy), was used. The collection of these legislations and guidelines was the background for the assessment scheme proposed in this paper (Table 2.) for evaluation of quality, hygiene, and safety. To reduce the effect of outlier sample values, two limits, *m* and *M*, were set representing good (<*m*), acceptable (between *m* and *M*) and not acceptable (>*M*) conditions. The percentage of samples that could hold values between *m* and *M*, were given for HPC and faecal indicator organisms, at 60% and 40%, respectively, whereas no samples should exceed *M*. Water used during production should hold potable quality, with no faecal indicator organisms, and HPC should not exceed *m*. No more than 40% of the seawater samples should be between *m* and *M*, concerning thermo-tolerant coliforms, whereas no seawater samples should exceed *m* for *Enterococcus*. No sample should contain *L. monocytogenes*.

Even though the legislations differentiate between primary producers, *i.e.* fishing vessels, and secondary producers, *i.e.* processing factories, the same assessment scheme was applied for all samples in this study. As this form provides recommendations only, any sample that fail to meet these recommendations, is not necessarily unfit for human consumption.

3. Results & discussion

During 41 samplings in the ten-year period covered in this work, 628 fish samples and 533 contact point samples were collected from fishing vessels and fish processing factories, summing up to 1161 samples.

3.1. Heterotrophic plate count and specific spoilage bacteria

A total of five hundred and ten (510) fish samples were assessed for heterotrophic plate counts (HPC), which varied widely within each sampling, as well as between sampling sites and fish species. The samples ranged from <3.0 log CFU/g, which was the detection limit for the applied method, to 7.4 log CFU/g. Figure 1 shows the frequency distribution and median values of the HPC and H_2S-producing bacteria. The HPC are used as a quality indicator, since increased bacterial numbers may indicate poorer storage conditions or improper handling. However, there are currently no internationally accepted guidelines for HPC in fish products, and the limit for good quality of fresh fish, which is set at $m < 5.7$ log CFU/g in the proposed assessment scheme, is based on the former Norwegian quality regulations for fish and fishery products (FOR-1996-06-14-667). Totally, 36% of the fish collected from vessels exceeded *m*, whereas 1.4% of the fish were above *M* (not acceptable). Although the latter fish make only a small fraction, the findings are still censurable considering the comparatively high HPC levels that early in the production chain. However, routinely performed washing with potable water, seemed to reduce the HPC, as only 12 of 120 samples (10%) from factories were exceeding *m*. These were all found during sampling #21 ($n = 12$), and no significant difference was found between the three fish samples collected from the landing tank, and the nine fish samples collected from the production line. However, when comparing all samplings at factories, the fish from the landing tanks had significantly lower HPC ($p = 0.0018$), than fish collected during production (Figure 2). This is somehow surprising as the water in these landing tanks,

Table 2
Outlined and proposed assessment scheme for fish-, surfaces-, and water samples. These guidelines are based on contemporary legislations for food products, and are set for quality, hygiene, and safety by limits of heterotrophic plate count (HPC), faecal indicator organisms (thermo-tolerant coliforms, and enterococci), and *L. monocytogenes*, respectively. All samples are evaluated according to good conditions (<*m*), acceptable conditions (between *m* and M), and not acceptable conditions (>*M*). The number of fish and surface samples with values between *m* and *M* should not exceed 60% and 40%, for HPC and faecal indicator organisms, respectively. No sample should be positive for *L. monocytogenes*. Water used in production should hold potable quality, with no faecal indicator organisms, and HPC should not exceed *m*.

Sample	Quality		Hygiene (faecal indicator organisms)				Safety	
	Heterotrophic plate count		Thermo-tolerant coliform		Enterococci		*L. monocytogenes*	
	m	*M*	*m*	*M*	*m*	*M*	*m*	*M*
Fish (log CFU/g)	5.7	6.7	0.6	1.3	2.7	3.1	neg	neg
Surface (log CFU/cm^2)	0.8	1.7	0.3	0.8			neg	neg
Water (log CFU 100 ml^{-1})	2 log CFU/ml		0	0	0	0	neg	neg

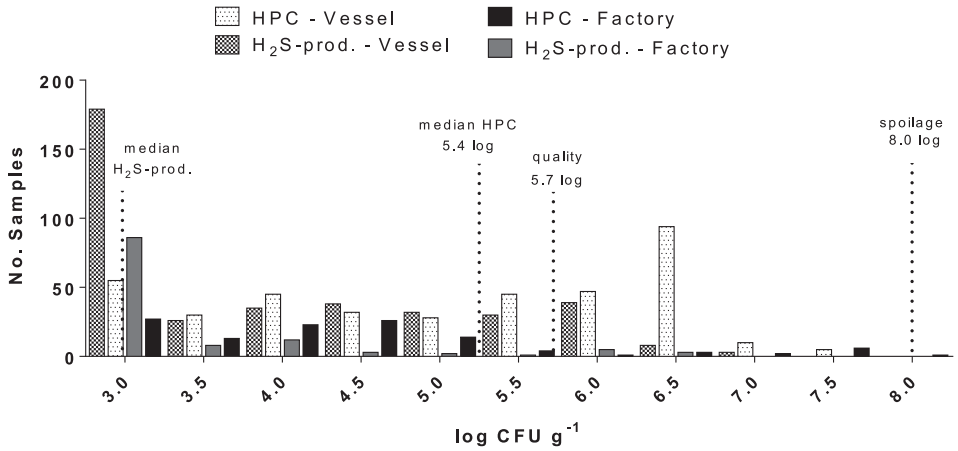

Figure 1. Number of samples given per 0.5 log value intervals (<3 to 8.0 log CFU/g) of HPC and H_2S-producing bacteria on board the fishing vessels and at the processing factories. The median of HPC and H_2S-producing bacteria, the good quality HPC limit (*m* = 5.7 log CFU/g), and the level at which to consider a fish spoiled (8.0 log CFU/g) (Gram and Huss, 1996), are shown with dotted lines.

containing either potable water or seawater, appears sludgy and higher HPC should be expected. The fish samples from the landing tanks also had significantly lower HPC (p = <0.0001) than fish collected from the RSW tanks at vessels. It seems that the exposure to water in the landing tanks reduce the HPC, although the fish are easily re-contaminated with bacteria during production, as generally seen during food production (Reij and Den Aantrekker, 2004).

High HPC is often related to increased number of spoiling bacteria, possibly reducing the self-life of the product. However, microbial spoilage of fresh fish stored aerobically under chilled conditions is commonly seen if the number of SSBs exceeds 8.0 log CFU/g (Gram and Huss, 1996). One of the samples in this study had such high values of H_2S-producing bacteria. Still, 20 samples had H_2S-producing bacteria between 6.0 and 6.8 log CFU/g, probably

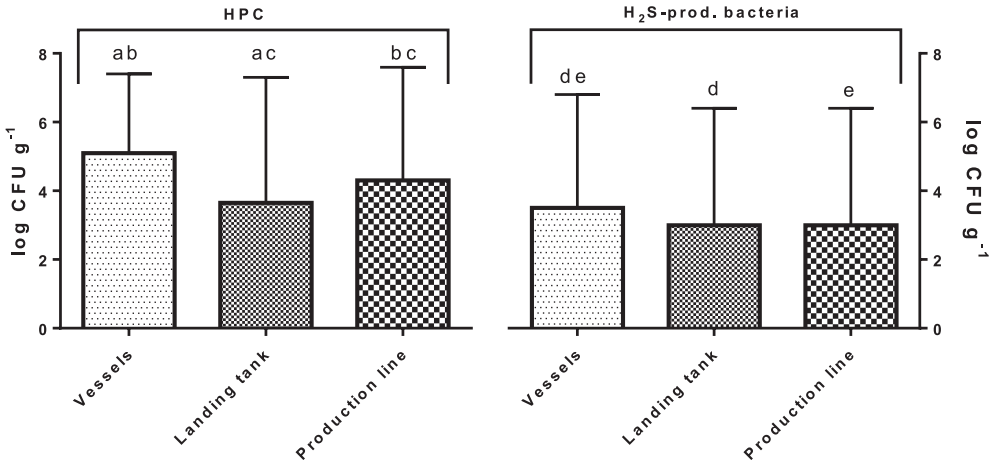

Figure 2. Log CFU g^{-1} (shown as median) of HPC and H_2S-producing bacteria of fish collected from fishing vessels (n = 390), landing tanks (n = 52) and production line (n = 68) at fish processing factories. Letters indicate significant differences (one-way ANOVA).

influencing the remaining shelf-lives of the end products. Of these 20 samples, 13 were from one sampling of blue whiting (#30), where the fish were caught by trawling and sold to a factory for production of frozen round fish intended for export. It is likely to assume that the fish have received a rougher treatment due to the trawling catch method, as compared to the remaining samples included in this paper. When comparing the fish samples from the vessels, landing tanks and production lines, the samples collected from the vessels only, differed significantly from the landing tanks ($p = <0.00001$) and those taken from the production lines ($p = 0.005$), with higher values of H_2S-producing bacteria.

Three hundred and thirty-five (335) contact point samples were collected from vessels (238) and processing factories (97), and analysed for HPC. Fifteen surface samples collected from the sift box, the sorting chamber and different tubes, had HPC values above *M* (not acceptable) according to the assessment scheme. Among these, 12 samples originated from two samplings collected before catch, indicating that the surfaces of the equipment were contaminated before the fish entered the vessel. At the processing factories, six samples from conveyor belts at two different samplings (#21 and #24), but the same factory, had values not acceptable (*M*). All surface values are plotted in Figure 3. Among the 59 water samples collected from vessels, 50 were above the detection limit of the applied method. Since these storage tanks contain refrigerated sea water (RSW), the samples are difficult to assess knowing that the number of cultivable bacteria in sea water depends on the location and could reach numbers between 4.0 log and 8.0 log CFU/ml (Austin, 1988). All water samples at the factories, collected from the landing tank and the washing tank were too numerous to count (TNTC) (>2.5 or > 3.5 log CFU/ml, dependant on the dilution). The present legislation for production water does not have an upper limit for HPC, but samples with more than 2.0 log CFU/ml, should be investigated (Council Directive 98/83/EC). In general, HPC of contact point samples are difficult to evaluate, as only small areas of the surface, or small amounts filtered water, are analysed, and do not necessarily reflect the cleanliness of the tested object. Small fractions of fish skin, mucus, and sludge might come along during sampling, though these residues might not be found across the whole test object, and the results could be inaccurate.

Table 3 gives an overview of all samplings that had HPC that were not in compliance with the assessment scheme.

3.2. Indicator organisms of faecal contamination

Among the 518 fish samples tested for coliforms (398 from vessels and 120 from factories), 25 samples showed values that were above detection limit (0.5 log or 1.0 log CFU/g). From sampling #32 fish were found positive for thermo-tolerant coliforms and presumptive *E. coli* (10 of 15 samples). The MPN values ranged between 0.6, which is acceptable (*m*) according to the assessment scheme, and 2.4 log CFU/g which is above *M* (not acceptable). Fish collected at factories were only tested for coliforms, and three samples were positive, including final products with values of 2.0 and 2.4 log CFU/g.

Three hundred and three (303) contact point samples were tested for Enterobacteriaceae and presumptive *E. coli*. Among the 115 samples collected from vessels and analysed for presumptive *E. coli*, four samples had values above *M* (not acceptable). One of these, collected from a sift box (#10) had values of 1.7 log CFU/cm^2, which indicates heavy faecal contamination. Among the 57 water samples collected from vessels, 15 were positive for *E. coli*, including five samples with values >1.7 log CFU 100/ml. These samples were collected from the RSW-tank at two vessels, during three different sampling (#20, #32 and #33). Equipment used on board fishing vessels, are often exposed to open air, and this increases the possibility for contamination from seawater and droppings from sea birds. However, the presence of *E. coli* indicates contamination from faecal matter and sewage (Noble et al., 2004).

In factories, fish and equipment are more likely to be exposed to human contact, and proper hygiene among the workers is crucial. In total, 89 contact point samples were analysed for Enterobacteriaceae and presumptive *E. coli*. Concerning samples of surfaces (71), *E. coli* was detected from a sorting machine (#9) and conveyor belts (#9 and #13) with values above *M* (0.8 log CFU/cm^2). Nine of the 18 water samples collected at factories were positive for coliform bacteria and five of these samples (#4, #6, #9, #13 and #23), were confirmed as presumptive *E. coli*, with values between 0.9 and 1.7 log CFU 100/ml. All values from surface samples tested for HPC, Enterobacteriaceae and presumptive *E. coli*, are plotted in Figure 3.

None of the 190 fish samples tested for enterococci was above detection limit (2.0 log CFU/g). However, 28 water samples collected from fishing vessels were positive for *Enterococcus*, and

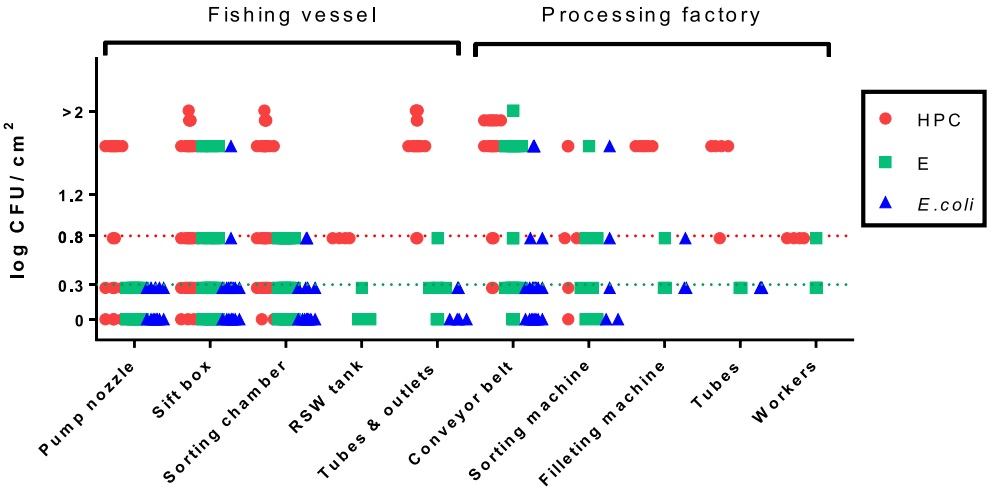

FIgure 3. Plot of Heterotrophic plate counts (HPC) (vessels + factories, n = 179 + 71), Enterobacteriaceae (n = 175 + 71) and *E. coli* (115 + 59) by Hygicult system TPC and E/β-gur, and contact plates (PC and VRBG), collected from surfaces at fishing vessels and fish processing factories. Good values according to the assessment scheme (Table 3) for HPC (*m*) and E/*E. coli* (*m*) is given by the dotted red line (0.8 log CFU/cm^2) and the dotted green line (0.3 log CFU/cm^2), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Overview of samplings with fish-, surface- or water samples that did not comply with the HPC limits in the proposed assessment scheme. The number of samples (n) run at each sampling, and the number of samples that had values that were acceptable (between *m* and *M*) or not acceptable (>*M*) are listed as *m*/*M*. Samples that were not in accordance with the assessment scheme are indicated in **Bold**.

Sampling#	1		2		3		4		6		9		11	
	n	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*
Fish	**8**	**2/2**	9	0	**4**	**3/0**	8	0	8	0	14	0	8	0
Surface	5	3/0	2	0	8	0	7	0	9	0	**5**	**5/0**	**5**	**4/0**
Water	**6**	**6**	**4**	**2**	**8**	**8**	**4**	**4**	**2**	**2**	**2**	**1**	**2**	**2**
Sampling#	**13**		**15**		**18**		**19**		**20**		**21**		**23**	
	n	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*
Fish	8	0	8	0	8	3/0	8	0	4	1/0	**12**	**3/9**	8	0
Surface	**4**	**4/0**	6	3/0	**12**	**12/0**	**7**	**7/0**	**6**	**6/0**	**10**	**9/1**	**9**	**9/0**
Water	**3**	**3**	**3**	**3**	**4**	**4**	**4**	**4**	**3**	**3**	**2**		**2**	**2**
Sampling#	**24**		**25**		**28**		**30**		**32**		**33**		**34**	
	n	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*
Fish	12	0	**50**	**25/1**	**50**	**50/0**	**20**	**15/5**	**20**	**18/0**	20	0/0	20	0/0
Surface	**13**	**7/5**									**14**	**4/7**	**14**	**9/1**
Water									**5**	**5**	**8**	**8**	**10**	**10**
Sampling#	**37**		**38**		**40**		**41**							
	n	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*	*n*	*m*/*M*						
Fish	20	0	**20**	**10/1**	20	0	20	0						
Surface water	**13**	**3/4**	6	3/0	**7**	**2/1**	**7**	**2/2**						

six samples (#3, #10, #12, and #32) had values between 2.0 and 3.0 log CFU 100/ml. As the enterococci have longer survival time in seawater (Noble et al., 2004), faecal contamination could be detected at a later stage, which might explain why the Enterobacteriaceae was not found in all of these samples. Concerning the 22 water samples collected from factories, 15 were positive for enterococci, with values ranging between 0.6 and 2.2 log CFU 100/ml. Four sample from the landing tank (#4 and #11), the washing tank (#11) and the sorting machine (#13) had values above 1.8 log CFU/ml.

Evaluating according to the proposed assessment scheme, two samplings, one factory (#13) and one vessel (#32), had fish samples that were contaminated with *E. coli*. At sampling #13, *E. coli* was detected at the conveyor belt, in water samples from the landing tank and from the sorting machine, thus *E. coli* was fairly distributed throughout the processing factory. At sampling #32, *E. coli* was detected in water samples from the RSW tank, both before and after capture, resulting in contaminated fish at time of delivery. This incidence has later been related to the location from where the RSW tanks were filled with seawater, an area commonly known for its poor quality due to sewage outlets. The crew at the vessel did not know this, thus filling of RSW tanks should be done far off shore to ensure clean seawater. Additional five samplings (#8, #9, #10, #11 and #33) had surface samples with too high numbers of presumptive *E. coli*, where the sorting chamber of vessels and the conveyor belts at factories seems to be the equipment most challenging to disinfect. An overview of all samplings that had values not according to the assessment scheme, is presented in Table 4.

3.3. Potential pathogens

Six hundred and five (605) fish samples were tested for *L. monocytogenes*, of which 450 samples were collected from fishing vessels. Among these, *L. monocytogenes* were found in six samples. Four of these positive samples were taken during sampling #5, while the other two came from sampling #20. Among the 155 samples collected from processing factories, only two were found positive for *L. monocytogenes*. This was one fish from the landing tank and one fish collected during production, both found at sampling #6. This factory had received fish from the vessel with the above mentioned positive sample (#5), showing that contamination of fish early in the production chain, could follow the product throughout the production. The transfer of *L. monocytogenes* from vessels to production facilities is of concern, as the pathogen is able to colonise food-processing environments. Moreover, *L. monocytogenes* is known to produce and establish in biofilms, persistent in the production environment and subsequently contaminate food products (Carpentier and Cerf, 2011; Cruz and Fletcher, 2011; Jensen et al., 2008; Møretrø and Langsrud, 2004; Pérez-Rodríguez et al., 2008). Additionally, *L. monocytogenes* is known to tolerate low temperatures, including freezing, which reduces the chance of eliminating these bacteria from the product (Rocourt et al., 2000). The occurrence of *L. monocytogenes* in fish from vessels and factories was less than 0.8%. This is lower than the findings reported in other studies. For example, 1.6% and 2.2% was reported by the Irish food safety department and EFSA, respectively (EFSA, 2013; Leong et al., 2014). In those cases the bacterium was detected on fish, the food safety authority was informed.

Three hundred and sixty-three (363) contact point samples (235 from vessels and 128 from factories), were tested for *L. monocytogenes*. From vessels, one sample of the pump nozzle at sampling #20, one water sample from the RSW tank at sampling #10, and one sample from the trawl bag at sampling #22, were found positive. Additionally, six surface samples collected from the pump nozzle, the sift box, and from four sites in the sorting chamber, and one water sample from the RSW tank, at sampling #5, were positive. Even though *L. monocytogenes* is found in the seawater environment, and can come along with the harvested fish, these samples were collected before catch, showing that the vessel already were contaminated. This might explain why the fish from this sampling was contaminated, as well as the fish delivered to the factory in sampling #6. A swab sample from a conveyor belt at this factory was also positive for *L. monocytogenes*, possibly contaminated by the fish that were delivered. However, the same conveyor belt, as well as water samples from the landing tank and the sorting machine, were found to be positive at two other samplings at the same factory (#9 and #13). One positive water sample from the landing tank was found at #23. It is well-known that the biofilm producing *L. monocytogenes* could be difficult to eliminate, once it has colonised an environment(Mizan et al., 2015). Floor drains in food processing facilities are typical sites for persistent *Listeria* sp.

Table 4
Overview of samplings with fish-, surface-, and water samples with presence of indicator organisms of faecal contaminations (*i.e.* thermo-tolerant coliforms and enterococci) at values that did not comply with the limit presented in the assessment scheme. The number of samples (n) run at each sampling, and the number of samples that had values that were acceptable (between *m* and *M*) or not acceptable (>*M*) are listed as *m/M*. Samples that were not in accordance with the assessment scheme are indicated in **Bold**.

Sampling#		1		2		3		4		5		6		7	
	Sample	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*
Tt. coliform	Fish	8	0	9	0	4	0	8	0	8	0	8	0	0	
	Surface	5	0	2	0	5	0	7	0	9	0	3	0	6	0
	Water	6	0	4	0	8	0	4	0	6	0	**2**	**1**	2	0
Enterococci	Fish			1	0										
	Water	**6**	**3**	**3**	**2**	**6**	**4**	**4**	**3**	**6**	**5**	**2**	**2**	**2**	**1**
Sampling#		**8**		**9**											
						10		**11**		**12**		**13**		**14**	
	Sample	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*
Tt. coliform	Fish	4	0	12	0	8	0	8	0	8	0	8		8	0
	Surface	**6**	**0/2**	**5**	**0/2**	**6**	**2/1**	5	2/0	6	0	**4**	**1/2**	6	0
	Water	2	0	**2**	**1**	2	0	2	0	1	0	**1**	**1**		
Enterococci	Fish														
	Water	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**2**	**2**
Sampling#		**15**		**18**											
						19		**20**		**23**		**32**		**33**	
	Sample	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*
Tt. coliform	Fish	8	0	8	0	8	0	4	0	8	0	**20**	**5/10**	20	0
	Surface	6	0	12	0	7	0	6	0	**9**	**8/0**				
	Water	2	0	**4**	**1**			**3**	**3**	**1**	**1**	**8**	**7**	**10**	**6**
Enterococci	Fish											20	0	20	0
	Water	**3**	**2**	**2**	**1**	**4**	**2**	**3**	**3**	**1**	**1**	**8**	**6**	10	0

and may be a source of contamination in the processing plant environment and possibly in food products (Zhao et al., 2006). Fourteen samples from drains were collected, but only one sample was positive (#11).

During this spot sampling, fish samples having *L. monocytogenes* were seen in three occasions (#5, #6 and #20). Furthermore, *L. monocytogenes*, was found on the surface of fishing gear and in water samples from the RSW tank, as well as on a conveyor belt at the factory. Additionally, samplings #9, #11, #12 and #13 had surface samples with *L. monocytogenes*, while samplings #9, #13, #22 and #23 showed positive water samples (Table 5.). According to current legislation, and the presented assessment scheme, no samples of fish or other food intended for consumption, without listericidal treatment prior to consumption, should hold *L. monocytogenes*. However, an EFSA report on zoonotic trends and food-born out-brakes, reports findings of *L. monocytogenes* in 18.6% of tested processing plants producing ready-to-eat fish and fishery products (EFSA, 2013).

The prevalence of coagulase positive staphylococci were examined in 32 fish samples from factories, and in 330 samples from vessels. One sample from one vessel (#28) were found to harbour coagulase positive staphylococci in a concentration of 1.0 log CFU/g. Several members of the genus *Staphylococcus* are potent toxin producers and are of concern in food preparation. Even though, these bacteria are not common in the marine environment and are recognised as competition weak (Götz et al., 2006), the presence in pelagic fish during export have been of concern in some situations. Thus, the prevalence of staphylococci have been included in our work.

Bacteria in the genus *Salmonella* were not detected in any of the 349 fish samples or any of the 99 surface samples examined in this study.

3.4. Assessment and guidelines

To better assess the microbiological quality, as well as the hygienic- and safety conditions of the present fish-, surface- and water samples of the spot-sampling programme, we introduced guidelines limits for good (*m*), acceptable (between *m* and *M*) and not acceptable (>*M*) values in the proposed assessment scheme (Table 2.). According to this scheme, 19 of 41 samplings included in this study, had all samples in compliance with the proposed guidelines. The presence of faecal indicator organisms and the pathogen *L. monocytogenes* in 21 and 9 samplings, respectively, suggests that the actual vessels and factories may improve the microbiological conditions through intensified cleaning and handling routines. At three particular samplings, a clear connection between the on-board environment and the fish under handling was evident. The presence of *E. coli* (#32) in both fish and contact point samples, indicate that the contamination were related to faecal bacteria in the RSW-tanks due to seawater of poor hygienic quality loaded into the tanks in near-shore waters. Additionally, *L. monocytogenes* were found in several samples (#5 and #6), and could tentatively be traced back along the production line.

Table 5
Overview of the samplings where *Listeria monocytogenes* was detected. As no samples should hold this bacterium (*m* and *M* = neg.), any presence of the bacterium was not according to the assessment scheme and is marked in **Bold**.

Sampling#	5		6		9		10		11		13		20		22		23	
	n	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*	*n*	*m/M*
Fish	**11**	**4**	**8**	**2**	14	0	8	0	9	0	9	0	**4**	**2**	23	0	16	0
Surface	**10**	**6**	**6**	**1**	**4**	**1**	**5**	**1**	**5**	**1**	**7**	**2**	**6**	**1**	21	0	10	0
Water	**6**	**1**	2	0	**2**	**1**	2	0	6	0	**3**	**1**			**3**	**1**	**3**	**1**

However, it is important to note that the number of incidents which involved marine fish as vehicle for human pathogens, is low (EFSA, 2015; Feldhusen, 2000; Painter et al., 2013).

In most quality or freshness issues concerning fish, it is the perception of the consumer, and not the actual bacterial numbers, that decide the acceptance of a given product. Fish products are more likely rejected by consumers before bacterial values that would indicate low quality or spoilage, are reached. In this study, the heterotrophic plate counts (HPC) are included as indicator of food quality, but indicate also clean surfaces and good water quality. If we evaluate all parameters collectively (quality, hygiene and food safety) according to the proposed assessment scheme, 34 of the 41 samplings (75%) had samples that did not comply with this scheme. Existing national or international legislations do not apply for all parameters included in the present study. Thus, by applying the herein proposed assessment scheme, we clearly showed that the microbiological conditions along the entire pelagic fish production chain, from handling and storage on-board the vessels to the final product, may be improved.

4. Conclusion

This paper introduces an assessment scheme for better evaluation of various microbiological quality, hygiene and safety parameters along the (Norwegian) pelagic fish production chain. Concerning hygiene and food safety, 51% of all fishing vessels and fish processing factories did not comply with the limits proposed in the assessment scheme. The overall evaluation, also including quality and cleanliness, showed that more than 75% of the vessels and factories examined involved samples that were not in accordance with the assessment scheme, and therefore have the potential to improve the fish handling routines, and subsequently the quality. Thus, controlling bacterial contamination is important all the way from catching and handling to processing, since bacteria that establish on the fish early during processing may retain throughout the production chain and adversely affect both quality and safety of the end product. Regular surveillance of the herein identified critical points along the pelagic fish production chain, based on assessment of the above parameters, are useful measures to optimise both quality and consumer safety of pelagic fishery products.

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