Exposure to Parvalbumin Allergen and Aerosols among Herring Processing Workers

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Background: There are increasing reports of allergies and respiratory symptoms among workers in the fish processing industry, coinciding with an increasing use of high-pressure water in the processing plants. However, few studies have measured exposure in these work environments.

Objectives: The aim of this study was to characterize the occupational exposure of workers to herring antigen and to screen environmental factors at a herring (Clupea harengus) plant in which new and more encapsulated filleting machines had been installed. To assist in this, a method to assess airborne exposure to herring allergen was needed.

Methods: Exposure to airborne herring antigen, mould spores, and endotoxin were measured during work. Antigen exposure was assessed using a newly developed sensitive (detection limit, 0.1 ng ml⁻¹) rabbit polyclonal sandwich enzyme-linked immunosorbent assay against the major herring muscle protein allergen, parvalbumin. Aerosols were measured by mass concentration (DataRAM) and number of particles (Climet I-500).

Results: Personal geometric mean herring allergen exposure was 986 ng m⁻³ at the old filleting workstations and 725 ng m⁻³ at the new workstations (difference not significant). Outside the production room, the level was ~130 ng m⁻³. Number of particles and mass concentration were both significantly lower around the new machines than around the old machines (P < 0.001 and P < 0.0001, respectively). The highest particle count was seen for the 0.3–0.5 μm fraction, with more than 400 000 particles per cubic metre air. Endotoxin concentration in the air varied between 3 and 92 EU m⁻³, with the highest levels when the catch mainly contained herring that had eaten krill or seaweed.

Conclusions: We developed a sensitive method to detect herring antigen. High exposure to herring antigen was measured during filleting work. The particles in the air around the fillet machines were mainly <0.5 μm and the newer encapsulated machines generated fewer particles. It is important to reduce occupational exposure of workers to aerosols by improving the ventilation system, machines, and organization of work.

Keywords: allergens; endotoxin; fish; measurements; moulds; particles

INTRODUCTION

Herring is the most commonly caught fish in Sweden, followed by cod and salmon (personal communication, Swedish Agency for Marine and Water Management). The processing of herring is likely to increase in magnitude in northern Europe, as this fish is not endangered and is regarded as healthy food from a cardiovascular point of view (Lindqvist et al., 2009).

As early as 1937, De Besche described how fish as an inhalant allergen can trigger asthma. Since then, many studies have described airway symptoms and asthma caused by exposure to allergens and irritating agents during handling and processing of fish and other seafood (Cartier et al., 2003).
The aim of this investigation was to survey the occupational exposure of workers at a herring processing plant in which new fish processing equipment had been installed in parallel with the older technology. We as others discovered that many fish factory workers have respiratory symptoms without specific sensitization to fish allergens. Therefore, we wished to undertake a workplace screening, measuring several potential environmental factors. To assess airway exposure to herring allergen, a further aim was to develop a sensitive method to measure the main identified allergen, herring parvalbumin.

MATERIAL AND METHODS

Workplace description

The fish processing plant, which employed ~40 workers, handled only herring (*Clupea harengus*). Six filleting machines (both new and old style) and the sorting table were located in a production room with an area of ~300 m² and no mechanical ventilation system (Fig. 1). Next to the sorting table was a door to the packing area, which was opened many times per shift. The old-style filleting machines were partly encapsulated and generated a visible fog over the line of machines, whereas the new machines were fully encapsulated. The workers used either a high-pressure sprayer or a hose to clean the machines and floor.

Fresh herring were delivered from contracted fishing vessels or tankers, with the fishing season beginning in August and continuing until May the following year. The quality of the herring varied with diet, season, and time since capture. If the herring had eaten krill or seaweed, their bellies were swollen and sometimes burst after capture.

On arrival at the plant, the herring were washed three times, sorted, and transported on a conveyor belt from the loading area into the production room with the filleting machines, where they were manually inspected, mechanically decapitated, and filleted. During each 8-h shift, each worker spent 3 × 2 h at the filleting machines, inspecting, and manually turning the fish, and 4 × 30 min at the sorting and inspection table, manually inspecting the fillets. Between May 2007 and April 2008, the average relative humidity in the production room varied between 65 and 93%, whereas the average temperature varied between 12.1 and 15.4°C.

Exposure measurements

Airborne herring protein and endotoxin concentrations were determined through personal aerosol sampling and area sampling. Measurements were performed seven times over the year. Sampling was performed for ~6 h at 2 l min⁻¹ airflow (n = 49 including six blank control filters for herring antigen analysis and n = 37 including five control filters for endotoxin). Antigens and endotoxin were collected on polytetrafluoroethylene (PTFE) filters (1 μm, Millipore FALP 02500) using IOM filter cassettes (SKC Inc., Valley View, PA, USA) fastened in the breathing zone. In 12 cases, workers carried double pumps for parallel sampling of herring antigen and endotoxin. Stationary pumps for area sampling were placed between the old filleting machines and the conveyor belts transporting the fillets to the sorting table, next to the sorting/inspection table, in the loading area, and in the packing area. After sampling, filters with antigen were stored at −20°C until extraction.

Mould spores in air were screened by placing one agar plate per 10 m² at a height of 1 m above the floor at different places in the factory (n = 8). Control plates were placed in the office and outside. The sampling time was 20 min. Sabouraud dextrose medium agar sedimentation plates were used (pH 5.8–6.0, 9 cm in diameter). Exposed agar plates were incubated at 25–30°C for 14 days, after which the yeast and mould colonies were counted. This method was used in order to screen for whether mould spores might be an important
exposure in this factory environment and motivate more advanced methods of analysis.

Gram-negative bacteria in air were screened by placing one agar plate at a height of 1 m above the floor at different places in the factory \((n = 12)\). Control plates were placed in the office. The sampling time was 20 min. Drigalski medium agar sedimentation plates were used (pH 5.8–6.0, 9 cm diameter). Exposed agar plates were incubated at 25–30°C for 2 days, after which Gram-negative bacteria colonies were counted. The agar plates were utilized to screen for whether Gram-negative bacteria might be an important exposure in this factory environment, so if present, we would analyse endotoxin, which is a more sensitive and expensive analysis. For standardized analysis of endotoxin in air \((n = 32)\), an endpoint LAL chromogenic test (Limulus Amebocyte Lysate-endosafe endochrome-K, Charles River Laboratories, Charleston, SC, USA) was utilized, with the standardized endotoxin method 1240, accreditation certificate number ISO 15189.

A stationary Climet I-500 was used to assess airborne particle size fractions. This is an optical particle counter instrument that counts particles in six different fractions: 0.3–0.5, 0.5–1.0, 1.0–5, 5–10, and >25 μm. The Climet I-500 was placed between the old machines and conveyer belts, between the new machines and conveyer belts and at the sorting table (Fig. 1).

Personal and stationary exposures to airborne particle sizes from 0.1 to 10 μm were measured by a person-carried DataRAM, Thermo pDR-1000AN (Personal Data logging Real-time Aerosol Monitor, Thermo Electron Corp., Franklin, MA, USA). The sampling period was 1 min, measuring average values over the sampling period. Measurements of aerosol concentrations were performed during 2 h. The measurement range was 1 μg m\(^{-3}\) to 400 mg m\(^{-3}\). The DataRAM is a real-time instrument based on passive nephelometric monitoring. Before each sampling day, the DataRAM was set to 0 with particle-free air recommended by the manufacturer. In addition, one

![Fig. 1. Schematic view of the production room with six filleting machines (1–6), machines 1–3 are old and 4–6 are new. The places where the workers stand are marked with stars. Cl denotes stationary measurement with Climet I-500. pDR denotes stationary measurement with DataRAM, Thermo pDR-1000AN. Filled ovals denote stationary measurement of airborne exposure to parvalbumin and endotoxin. From the filleting machines, the herring are transported on a conveyer belt to the sorting and inspection table in the control section where the workers inspect the fillets. The fillets are then transported to the packing area outside the production room. Under the conveyer belt, there is another conveyer belt that transports the leftover parts of the herring out from the production room.](https://academic.oup.com/annweh/article-abstract/57/8/1020/147344)
DataRAM was placed in front of the old and new machines next to where the person worked (Fig. 1).

Both Climet I-500 and DataRAM were used to screen the particles and check the ventilation.

Development of herring parvalbumin enzyme-linked immunosorbent assay

An assay to measure herring antigen was developed by preparation of the antigen, immunization of rabbits, purification of antibodies, and setting up the enzyme-linked immunosorbent assay (ELISA) according to strict quality criteria. Herring (C. harengus) parvalbumin, a muscle protein and a major allergen (Van do et al., 2005), was purified at the University of Bergen from a crude herring extract using anion-exchange chromatography on a diethylaminoethyl cellulose column. After washing with 100 mM phosphate buffer (pH 5.4) and elution diethylaminoethyl cellulose column. After washing with 100 mM phosphate buffer (pH 5.4) and elution with 150 mM phosphate buffer, the parvalbumin-containing fraction 2 was dialysed with 50 mM NH₄HCO₃ and freeze dried. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis showed a strong parvalbumin band at 12 kD with some weak minor bands at larger molecular weight.

Rabbit polyclonal antibodies against parvalbumin were prepared at the University of Bergen. Four rabbits (Crl:KBL-NZW) were immunized four times with herring parvalbumin in complete (immunization I) or incomplete Freund’s adjuvant (II–IV). Immunization was performed by an authorized animal technician. After 16 weeks, the animals were sacrificed and finally rabbit antiserum was collected and stored at −80°C until use.

Gel filtration of rabbit antisera was performed by separation of the 25 ml pools by molecular-sieve chromatography on two series-coupled columns (2.6 × 60 cm Superdex 75). The fractions containing immunoglobulin G (IgG), as analysed by dot blot using labelled swine anti-rabbit IgG, were then pooled and further fractionated by ion-exchange chromatography on a 1.6 × 25 cm ResQ column. The IgG-containing fractions were identified as before using labelled swine anti-rabbit IgG and pooled for further purification by affinity chromatography.

The two rabbit sera (rabbits 3 and 4) with the highest titres to parvalbumin were chosen for affinity purification of rabbit anti-herring antibodies, performed using a 2 ml AminoLink Plus column (Pierce Biotechnology Inc., IL, USA, article 44894) according to the manufacturer’s instructions. Briefly, 10 mg herring parvalbumin antigen was coupled to the column, followed by blocking the binding sites of the column and thorough washing. The rabbit antibodies were passed through the column and allowed to bind. After extensive phosphate buffered saline (PBS) washing, the antibodies were eluted using 0.1 M glycine–HCl, 0.5 M NaCl at pH 2.5, followed immediately by neutralization to pH 7.0 with NaOH. The affinity purified antibody preparation buffer was changed using extensive dialysis in PBS.

For use in the herring parvalbumin ELISA, a portion of the affinity purified rabbit antibody preparation was labelled with biotin using EZ-Link Sulfo-NHS-LC-biotin (Pierce, IL, USA) according to the manufacturer’s instructions.

Analysis of herring antigen in samples

Antigens were extracted from the filter during rotation in 2 ml PBS, 0.5% Tween, 0.15% Kathon CG™ at room temperature for 2 h. The eluate was centrifuged for 10 min at 1000 g to remove debris. Bovine serum albumin (BSA) was added to 1% and the eluates were aliquoted and kept at −20°C until analysis.

In the herring ELISA, Nunc MaxiSorp 96-well plates were coated overnight with 5 μg ml⁻¹ affinity purified rabbit polyclonal antibodies against herring parvalbumin. The plates were blocked for 1 h using PBS, 1% BSA, and washed four times in PBS, 0.05% Tween. Duplicates of standard dilutions of herring parvalbumin determined using Bicinchoninic acid (Pierce) at 0.1–10 ng ml⁻¹ and of workplace samples diluted from 1/10–1/1000 were added and the plates were incubated at ambient temperature for 2 h. After washing, biotinylated rabbit antibodies against parvalbumin were incubated for 1 h. Bound antibodies were detected after washing using a streptavidin–alkaline phosphatase (Mabtech AB, Stockholm, Sweden) and visualized using TMB (3,3′,5,5′-tetramethylbenzidine) (K-Blue Neogen, NY, USA), stopped with 1M SO₄ after 15 min, measured at 450 nm (absorbance at 650 nm subtracted) using a SpectraMax plate reader, and analysed with 4-PM curve fit including standard deviation (SD) and coefficient of variation (CV) % calculations using SoftMax software (Molecular Devices, CA, USA).

Statistical analyses

Descriptive statistics were expressed as either arithmetic means (AM) or geometric means (GM) with SD. For exposure of airborne allergen, GMs with SD were calculated since the data were not normally distributed. For particle concentrations, both AMs with 95% confidence intervals (CIs) and GMs with geometric standard deviation (GSD) were calculated.

We also applied time-series analysis (Proc ARIMA) to estimate average particle concentration
(numbers of particles per cubic metre), since the particle measurement data consisted of several different measurement episodes that were correlated with each other. Time-series analysis helped us deal with this autocorrelation problem. The distribution of particle counts was monitored graphically.

All statistical analyses were performed using version 9.2 of the SAS software package and \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**ELISA method**

A sensitive sandwich ELISA method was developed (detection limit: 0.1 ng herring parvalbumin per millilitre). The quality criteria for the assay were stringent: low background, \(<0.2\) optical density (OD); only standard curve values between 0.05 and 3 OD above background were used; a high curve fit, \( r^2 > 0.99 \); and CV < 10% for all duplicate standards and samples. A typical herring parvalbumin standard curve is shown in Fig. 2.

The purified rabbit antibodies were tested for cross-reactivity to parvalbumins from salmon, cod, and pollack, as well as five indoor allergens (Indoor Biotechnologies, VA, USA), and mouse and rat urinary antigens. Antibodies raised to herring parvalbumin recognized salmon, pollack, and cod parvalbumins, giving nominal allergen values that were 18, 32, and 32% of the added amounts, respectively, whereas no cross-reactivity was found to indoor, mouse, or rat allergens. All six control filter samples were below the detection limit.

**Herring parvalbumin exposure**

Two filters were deleted due to splashing and clogging of the filter during sampling. Herring parvalbumin levels in air varied between 63 and 9800 ng m\(^{-3}\) (median: 630 ng m\(^{-3}\), GM: 588 ng m\(^{-3}\)). The production workers worked both at the filleting machines and at the control/inspection table during a shift. The GM of herring allergen exposure measured as personal exposure during such a shift was 986 ng m\(^{-3}\) with the old filleting workstation (\( n = 14 \) samples) and 725 ng m\(^{-3}\) at the new workstation (\( n = 11 \); no significant difference; Fig. 3A). Levels for workers whose shifts were placed outside the production room, at the station for loading herring, and in the packing area were lower (GM: \(~130\) ng m\(^{-3}\)).

**Endotoxin exposure**

Since gram-negative bacteria were found in various amounts around the old and new workstations (150–2000 CFU per agar plate), we sampled and analysed exposure to airborne endotoxin. No living Gram-negative bacteria were found in the office.

![Standard Curve](https://example.com/standard_curve.png)

**Fig. 2.** Typical herring parvalbumin ELISA standard curve. Parvalbumin standards between 0.1 and 10 ng ml\(^{-1}\) are added in duplicates. Points show mean (SD) optical density (OD) values at 450 nm after subtraction of background (wells w/o added antigen). Analysis using Softmax 4-parameter curve fit, \( r^2 = 1 \).
Endotoxin concentration in the air varied between 3 and 92 EU m\(^{-3}\) (median: 11.6 EU m\(^{-3}\), GM: 15.6 EU m\(^{-3}\), Fig. 3B). The highest levels were found when the catch mainly contained herring that had eaten krill or seaweed. Low amounts of endotoxin in air were found in the loading and packing areas (median: 1.2 EU m\(^{-3}\), GM: 2.5 EU m\(^{-3}\)). There were no significant differences between the old and the new workstations with regard to personal exposure to endotoxin.

**Mould exposure**

Low amounts of mould spores in the air (a mean of 2 colonies per plate) were found around the loading area, the old and the new machines, and the packing area, lower than the outside milieu (10 colonies per plate). No yeast spores were found. Repeated samplings of mould spores showed the same results.

**Particle exposure**

The Climet I-500 measured stationary different airborne particle size fractions. Older filleting machines gave a significantly higher exposure to particles compared with newer machines \((P < 0.001)\). The particle fraction with highest particle count through all seven measurements was the 0.3–0.5 \(\mu\)m fraction, which showed more than 400 000 particles per cubic metre air. The >10 \(\mu\)m fraction showed <5000 particles per cubic metre air and the >25 \(\mu\)m fraction showed <100 particles per cubic metre air (Fig. 4). The Climet CI-500 has an internal battery that was discharged without warning during measurement on 2 October at the old machine, so the data were destroyed.

Real-time DataRAM personal aerosol exposure was measured as mean concentration. Personal GM exposure during mean time of 2 h at the old machine was 0.24–2.09 mg m\(^{-3}\) at the old machine and 0.12–0.72 mg m\(^{-3}\) at the new machine (Table 1). Personal exposure at the old machine was significantly higher than that at the new machine \((P < 0.0001)\) and there was a significant difference between stationary measurements at the old and new machines \((P < 0.0001)\).

**DISCUSSION**

In previous studies of the occupational environment in the fish processing industry, we and others have found that many workers have respiratory symptoms without specific sensitization to fish allergens (Jeebhay and Cartier, 2010; Dahlman-Högglund et al., 2012). Furthermore, new machinery is being installed in fish factories, with unknown effects on worker health. To undertake a workplace screening of a herring factory that had invested in new filleting machines, we, therefore, measured several environmental factors that could potentially affect the workers.

We developed a new sensitive herring sandwich ELISA to detect airborne herring antigen. Workers were highly exposed to parvalbumin during handling and controlling of the herring fillets. Levels of herring parvalbumin were nearly an order of magnitude lower in the loading and packing areas compared with the filleting and controlling/inspection workstations. No significant differences for either herring antigen or endotoxin were found over a work shift between
workers who had worked with the new filleting machines and those who had worked with the old. However, when measuring particle exposure during 2 h of work stationed at either the new filleting machines or the old filleting machines, the levels differed significantly. Our study is the first to measure allergen, mass concentration, and number of particles in a herring plant. The particle fraction with highest particle count through all seven measurements was 0.3–0.5 μm fraction.

Our assay used polyclonal affinity purified antibodies from rabbits against the main identified allergen, herring parvalbumin, (Bugajsk-Schretter et al., 1998). Parvalbumin is a calcium-binding muscle protein with a molecular weight around 12 kD, with high homology between many fish and amphibian species. Cross-reactivity in tests among species has been found previously, both by us and by other researchers (Lindström et al., 1996; Hilger et al., 2004; Dahlman-Höglund et al., 2012). Parvalbumin is a relevant antigen to measure, as ~95% of fish allergic subjects are sensitized to parvalbumin and it is present in comparatively large amounts in herring, constituting 3% of total protein in raw herring muscle tissue (Kuehn et al., 2010). We applied stringent assay quality control criteria and the high sensitivity of the assay allowed work samples to be diluted at least 10-fold.

Other studies from finfish production have measured allergen concentrations in the range of 0.002–5.100 μg m$^{-3}$ air (Jeebhay and Cartier, 2010), using different techniques. In a previous study from a herring factory in Norway, production workers were exposed to measurable herring mean levels (three samples) of 0.9 μg m$^{-3}$ (Bang et al., 2005). However, exposure was assessed using a patient IgE inhibition ELISA method, so it is not possible to compare these levels with those measured in the present study using a polyclonal sandwich ELISA. Indeed, in previous comparisons between inhibition and sandwich setups, differences of orders of magnitude have been observed in nominal allergen levels derived from samples analysed by different laboratories (Renström et al., 1997; Hollander et al., 1999).

When comparing results from different studies, it is also important to keep in mind that different
Exposure among herring processing workers

Plants have different ventilation processes, different production rates, and different ways to handle herring. With the polyclonal sandwich ELISA developed in the present study, it is possible to compare job tasks in different herring plants.

Mould spores were screened, for whether mould might be an important exposure in this factory environment, and motivate more advanced bioaerosol techniques of analysis. However, few mould spores were found. Thus, for these workers, mould probably does not contribute greatly to the health risk. Since gram-negative bacteria were found in all of the agar plates in the production room, we sampled and analysed exposure to airborne endotoxin.

In the measurements of endotoxin during herring processing, the highest values were found in the personal measurements, but levels varied widely among samples. The values also varied with the quality of the herring. Highly variable airborne endotoxin levels have also been shown in other studies from processing plants (pilchard, cod, and cod plus anchovy; Jeebhay and Cartier, 2010). Furthermore, the endotoxin levels were mostly fairly low, at least compared with other animal handling facilities such as swine confinement buildings (Liebers et al., 2006). However, as is the case with allergen measurement methods, nominal values may depend upon sampling, elution, and analysis factors (Liebers et al., 2006). In addition, peak exposures may be more clinically important than mean exposure level over a work shift.

When sampling for endotoxin in this study, we used PTFE filters. Bang et al. (2009) measured higher concentrations of endotoxin (mean: 103.7 EU m$^{-3}$) using glass fibre filters in a herring factory, whereas Spaan et al. (2007) showed that the type of filter had a large effect on the results. After finishing the data collection for the present study, we tested both PTFE and glass fibre in the herring plant and found that the glass fibre filters showed slightly higher values than PTFE filters. Thus, the endotoxin measurements in this study may be underestimated.

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<th>Date</th>
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<th>GM</th>
<th>GSD</th>
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Table 1. Particulate mass concentration measured with DataRAM mg m$^{-3}$.

Particle size range: 0.1–10 μm. Particles were measured on workers personal at old/new machines. Particles were measured stationary at old/new machines.

Liebers et al. (2006) measured higher concentrations of endotoxin (mean: 103.7 EU m$^{-3}$) using glass fibre filters in a herring factory, whereas Spaan et al. (2007) showed that the type of filter had a large effect on the results. After finishing the data collection for the present study, we tested both PTFE and glass fibre in the herring plant and found that the glass fibre filters showed slightly higher values than PTFE filters. Thus, the endotoxin measurements in this study may be underestimated.

In a study of endotoxin distribution on particles of different sizes in straw storage halls, the highest concentration of endotoxin was found in the thoracic fraction of dust, both when considered per milligram of dust and per number of particles.
(Madsen and Nielsen, 2010). Other studies have shown that endotoxin fragments stick preferentially to fine particles of 0.04–0.37 μm (Reponen et al., 2005; Wang et al., 2007). Due to their small size, fine particles have a longer residence time and penetrate deeper into the respiratory system (Löndahl, 2009).

Only a few studies have addressed environmental particle exposure in finfish (salmon, Pollack, and pilchard) processing environments (Jeenehay and Cartier, 2010). In these studies, the particulate mass concentration in the respirable fraction ranged from 0.04 to 3.6 mg m\(^{-3}\) air. In fish processing, a great deal of water is used in different processes (degutting, decapitation, cooking/boiling, and cleaning of the processing line), which will influence the aerosols in the work environment. The way in which the workers use this water (e.g., using a high-pressure sprayer) will influence the size and lifetime of the particles. It is, therefore, important to measure different particle characteristics when discussing health effects, for example by measuring the mass concentration of particles >0.5 μm. When counting ultrafine particles (<0.1 μm), the number of particles is generally a good metric. Since we did not know the characteristics of the particles in the setting for this study, we measured both mass concentration and number of particles. The particle counter we used did not count ultrafine particles. The particle fraction on Climet with the highest particle count through all seven measurements was the 0.3–0.5 μm fraction. The newer machines were more encapsulated than the older machines, which resulted in significantly lower concentrations of particles around the new machines in both personal and stationary exposure measurements. These results show the importance of preventing aerosol spread by encapsulating the machine. Before the project started, The pilot study day was performed. The pilot study day the workers complained about the quality of air and some workers had upper respiratory problems during work; the levels of aerosols were found to exceed the Climent instrument’s measuring range. They had been using a high-pressure sprayer; we advised them to reduce the use of this sprayer and use a hose instead during cleaning.

When using these instruments to screen particles in an working environment with high humidity that cause hygroscopic growth (Lee et al., 2008), condensation may occur that could affect the optical and light scattering detector on ClimentI-500. When using DataRAM in environments with relative humidity >60%, the data should be corrected using a correction factor, since the instrument overestimates the particle mass at high relative humidity (Chakrabarti et al., 2004). Here, this was not done since the comparisons are made between measurements from the same day (when the relative humidity is assumed to be constant during the measuring period). With our results, the plant could improve their ventilation in the production room.

The workers at the herring plant reported feeling worse during the night shift when the day shift workers had cleaned the floor with a high-pressure sprayer or when the herring had eaten krill. When herring have eaten krill, their bellies become swollen after capture. Sometime the whole catch could contain herring that had eaten krill. The fisherman noticed directly and transported the herring as soon as possible to the plant since it affected the quality of meat. The fillet machine could not fillet the herring in normal way because the meat was softer so pickled herring was produced.

A recent study (Larsen et al., 2008) showed that purified salmon trypsin in vitro can activate human pulmonary epithelial cells to induce secretion of the pro-inflammatory cytokine interleukin 8. The authors suggested that inhalation of particles containing biological material such as salmon trypsin could produce inflammatory effects in the airways and showed that water samples from the salmon industry had high enzymatic activity. Another study from the same group demonstrated that fish tissues contain inflammatory potential linked to serine protease activity (Bang et al., 2009). Bønløkke et al. (2004) have suggested that rinsing water in herring plants can also contain pro-inflammatory agents other than endotoxin. In addition, other factors in the environment could cause work-related airway symptoms, for instance gram-positive bacteria, which we have not measured.

Our recommendation to the herring plant workers to reduce their use of the high-pressure sprayer resulted in a reduced exposure to fine bioaerosols. There is a need for further research addressing the fine particle content in the air of finfish plants.

In conclusion, we have developed an ELISA to measure airborne exposure to herring parvalbumin, a major herring allergen. We have characterized the particle exposure of particles in the air around the filleting machines in a herring plant and showed that most of the particles are <0.5 μm and that the newer encapsulated machines generate fewer particles.
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REFERENCES


