Occupational Exposure to Bioaerosols in Norwegian Crab Processing Plants

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ABSTRACT

Introduction: Aerosolization of components when processing king crab (Paralithodes camtschaticus) and edible crab (Cancer pagurus) may cause occupational health problems when inhaled by workers.

Methods: A cross-sectional study was carried out in three king crab plants and one edible crab plant. Personal exposure measurements were performed throughout work shifts. Air was collected for measurement of tropomyosin, total protein, endotoxin, trypsin, and N-acetyl-β-d-glucosaminidase (NAGase). T-tests and ANOVAs were used to compare the levels of exposure in the different plants and areas in the plants.

Results: Total protein and tropomyosin levels were highest in the edible crab plant, endotoxin levels were highest in king crab plants. King crab exposure levels were highest during raw processing. Tropomyosin levels were highest during raw king crab processing with geometric mean (GM) 9.6 versus 2.5 ng m⁻³ during cooked processing. Conversely, edible crab tropomyosin levels were highest during cooked processing with GM 45.4 versus 8.7 ng m⁻³ during raw processing. Endotoxin levels were higher in king crab plants than in the edible crab plant with GM = 6285.5 endotoxin units (EU) m⁻³ versus 72 EU m⁻³. In the edible crab plant, NAGase levels were highest during raw processing with GM = 853 pmol4-methylumbelliferone (MU) m⁻³ versus 422 pmol4-MU m⁻³ during cooked processing. Trypsin activity was found in both king crab and edible crab plants and levels were higher in raw than cooked processing. Differences in exposure levels between plants and worker groups (raw and cooked processing) were identified.

Conclusions: Norwegian crab processing workers are exposed to airborne proteins, tropomyosin, endotoxins, trypsin, and NAGase in their breathing zone. Levels vary between worker groups and factories.

KEYWORDS: bioaerosol quantification; crab processing; endotoxins; NAGase; occupational health; protein; tropomyosin; trypsin

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INTRODUCTION

Workers processing seafood are exposed to bioaerosols in their breathing zone that may be inhaled (Weytjens et al., 1999; Bang et al., 2005; Shiryaeva et al., 2014). Bioaerosols are particulate matter or liquid droplets suspended in air, containing agents of biological origin such as endotoxins, microorganisms, and proteins including high molecular weight allergens and enzymes (Jeebhay et al., 2005; Bang et al., 2005) depending on the type of seafood being processed (Jeebhay et al., 2001, 2005). Several steps involved in processing crab, e.g., butchering, degilling, cracking, boiling, and washing/scrubbing, are shown to produce aerosols (Jeebhay et al., 2001; Jeebhay and Cartier, 2010; Jeebhay, 2011). Previous investigations show that a considerable portion of airborne particulate mass produced during crab processing is within respirable range (Jeebhay et al., 2001). Adverse health effects from bioaerosol exposure (Jeebhay et al., 2008; Gautrin et al., 2010; Eduard et al., 2012; Shiryaeva et al., 2014; Watanabe et al., 2016) such as immunological sensitization, respiratory symptoms, bronchial hyper-responsiveness, and occupational asthma have been found in exposed seafood workers. Sensitization is documented in workers processing fish, mussels, prawns, and crabs. Workers in the shellfish industry seem to have more severe symptoms and are more often sensitized than those in the fish industry (Jeebhay et al., 2001; Thorn, 2001; Cartier et al., 2004; Jeebhay and Cartier, 2010; Shiryaeva et al., 2010; Lopata and Jeebhay, 2013).

Total protein levels are often measured in exposure studies. Such data do not describe the individual protein components, but give an indication of the total load of proteins inhaled by the workers. Adverse health effects or a dose–response relationship between levels of different components of bioaerosols and respiratory symptoms, allergy, and asthma are found in seafood workers, including snow crab workers (Jeebhay et al., 2001; Gautrin et al., 2010; Jeebhay, 2011; Lopata and Jeebhay, 2013). Lopata and Jeebhay (2013) listing current seafood allergens, showing tropomyosin from crab meat as one of the main allergens causing occupational allergy. Endotoxin, a well-known proinflammatory mediator, has been confirmed to be present in crab processing plants (Neis, 2004), but exposure levels have not been measured previously. Chitin, a polymer of β-(1–4)-linked N-acetylg glucosamine (NAG) is present in the shell of crustaceans (Chen et al., 2011). Since NAGase is an enzyme known to digest chitin, it is expected to be present in the bioaerosols during crab processing. NAGase can stimulate exposed cells to secrete inflammatory mediators such as interleukin-8 (Allermann et al., 2006). Trypsin is a crucial and widespread digestive enzyme, catalysing protein hydrolysis in vertebrates as well as invertebrates. Trypsin exposure is linked to innate inflammatory responses in cell models and is also suspected to play a role in allergy, including seafood allergies (Baur, 2005; Larsen et al., 2008; Sun and Lopata, 2010). King crab trypsin has been shown to be a potent stimulator of protease-activated receptors linked to inflammatory reactions in airway cell models (Larsen et al., 2011).

Previous studies on crab processing workers have mainly focused on health outcomes and lack data on bioaerosol exposure levels and content. Variations between processing plants such as building parameters and processing technology (plant effect), may contribute to differences in exposure levels. Technologies used in the current plants ranged from mainly manual work with simple tools to modern, highly automated processing lines which will affect bioaerosol production and content (Moody et al., 1993; Stellman and Office, 1998). Important factors affecting the generation and content of bioaerosols are placements of ventilation systems and the proximity of workers to machines. Other important factors such as shielding of work tasks, areas producing high levels of bioaerosols, use of high pressured air, or water hoses (Gaddie et al., 1980) along the process lines and during general cleaning play a major role in workers’ exposure.

The aim of this study was to explore the levels of important agents released during the processing of king crab and edible crab in Norwegian crab processing industries. The knowledge gained may form a necessary base for guidelines and advice to plant management to improve workplace design, procedures, and personal protective equipment. This can be used to reduce bioaerosol exposure and health problems among crab processing workers.

MATERIALS AND METHODS

This study is a cross-sectional study with personal air samples taken from workers’ breathing zone in three king crab and one edible crab plant on the North Norwegian coastline between November 2009 and
October 2011. Written informed consent was obtained from all participants. The study was approved by the Regional Committee for Medical Research Ethics in Northern Norway, Tromsø.

Plants
A graphical illustration of the plant layouts are presented in Fig. 1. The three king crab plants in Fig. 1A–C and the edible crab plant in Fig. 1D.

Edible crab (Cancer pagurus) in Norway is processed in a few land-based plants. One well established plant built for edible crab processing was included in this study. The plant layout is optimized for crab processing with separate areas for raw and cooked crab. The processing line is streamlined so workers stand close together. Automated brushes rotating at high speed and transportation belts keep workers stationary. Consequently, their exposure should be quite stable through their shift.

The king crab (Paralithodes camtschaticus) industry expanded rapidly in the coastal areas of North-Eastern Norway when commercial fishing started in 2002. Many changes in stock migration and regulated capture quotas have resulted in crab processing often being a side-line for land-based fish processing plants. Since king crab plants are often small and not built for crab processing, the processing equipment is placed in the plant during crab season and removed when the season is over. The processing lines have a compact layout and are not optimally placed. Workers often do different work tasks during their shifts.

Workers were subdivided according to whether they were processing mainly raw or cooked crab, or

Figure 1. Factory layouts of three different king crab factories (A–C) and one edible crab factory (D). Walls and doors separating areas are drawn as solid lines. Rippled lines indicate areas of production. Work tasks are as follows (not all tasks were present in all plants); (1) cracking, (2) de-gilling and cleaning raw crab, (3) packing raw crab for cooking, (4) cooking, (5) processing cooked crab (packing, sorting, mincing shell and muscle), (6) varying work tasks in all areas of the factory (transport of crabs or vats on trucks, cleaning vats or plant, maintenance).
performing work tasks in both raw and cooked king crab during the same shift such as truck driving or cleaning. This subdivision reflects how the work was organized in the crab factories.

**Study population**

Workers were recruited in collaboration with the plant management. At each plant, randomly selected production workers voluntarily carried sampling equipment during their work shifts. In total, 45 people participated in the study; 33 king crab workers (8 female) and 12 edible crab workers (3 female).

**Exposure measurements**

Personal exposure measurements were performed throughout the work shifts using SKC Sidekick (SKC Ltd., Dorset, UK) sampling pumps. Air flow rates were set to 3.0 L min⁻¹ for tropomyosin, total protein/trypsin and NAGase to ensure enough bioaerosols were collected, and 2.0 L min⁻¹ for endotoxin as it was recommended by the supplier of the sampling head. Each worker randomly carried a backpack containing two or three pumps each connected to a filter cassette. One filter cassette was analysed for total protein and trypsin, one for tropomyosin, and the third for either endotoxin or NAGase. The air samples were collected when the shift ended or after 8 h if the shift lasted longer. The flow rate for each pump was calibrated before and after collection using Bios Defender 520 (SKC Ltd., Dorset, UK) and the sampling times (minutes) were registered. If the flow deviated with more than 10% from the start of shift, the samples were discarded. The mean air volume collected was 872.4 L (range 40–1440 L) for king crab and 1173 L (range 97.5–1578 L) for edible crab. Filter cassettes were placed in the workers’ personal breathing zone. For tropomyosin, total protein/trypsin and NAGase, SureSeal Air Monitoring Cassettes (37 mm, 3-pc, styrene SKC Ltd., UK) were used. For endotoxin, PAS6 cassettes (Personal Air Sampler with 6 mm inlet) manufactured at the STAMI National Institute of Occupational Health in Oslo, Norway were used. Protein/trypsin and tropomyosin samples were collected on polystyrene filters (Whatman GF/A, Kent, Maidstone) and NAGase samples on PC (polycarbonate) filters. At break times, the pumps were stored away from the production area to prevent overestimation of exposure, but kept running to represent the mean exposure during the shift, including time away from the production area. Whether workers were processing raw or cooked crab during the shifts was registered. Within an hour after sampling, the external surfaces of the SureSeal Air Monitoring Cassettes were cleaned with 70% ethanol to remove external contaminations before transport and extraction. Tropomyosin, total protein/trypsin and NAGase cassettes were stored at −20°C in the field and at −70°C in the lab. Endotoxin cassettes were stored at +4°C until extraction. Exposure results were calculated as time weighted averages. Field blanks and analytical blanks were included in all analyses.

During sampling, 78 samples of tropomyosin, 67 samples of total protein, 15 samples of endotoxin, 36 samples of trypsin and 13 samples of NAGase were collected. The difference in sample numbers were due to different technical problems.

**Total protein analysis**

The protein filters were extracted in 1.0 ml PBS with 0.05% Tween20. Samples were transferred to mini eppendorf tubes and stored at −70°C. Manual QuantiPro BCA Assay Kit (Sigma-Aldrich, St. Louis, USA) was used to determine total proteins in filter extracts (μg ml⁻¹) in the samples by colorimetric reading of Cu⁺⁺–BCA complex in a spectrophotometer at 560 nm. Air levels (μg m⁻³) were calculated from these values by consideration of air flow through the filters and sampling time. Analyses were performed at the Department of Medical Biology at UiT the Arctic University of Norway.

**Tropomyosin analysis**

Exposure to the major crab allergen, tropomyosin was quantified by a method described by Kamath et al. using a capture ELISA method (Kamath et al., 2014). The filters were extracted in 1.0 ml PBS with 0.5% Tween 20 and NaN3 for conservation, transferred to mini eppendorf tubes with bovine serum albumin (BSA) and kept frozen until analysed. Purified recombinant tropomyosin was used as the allergen standard. A 96 well high binding Costar microtitre plate (Sigma Aldrich, USA) was coated with anti-tropomyosin antibody in carbonate buffer, pH 9.6 and incubated overnight. After blocking the wells with Pierce Superblock
buffer (Thermo Fisher, Melbourne, Australia) the standards, blank and diluted or undiluted filter extracts were added to the wells and incubated at room temperature. After washing with phosphate buffered saline, pH 7.2 with 0.05% Tween-20, the wells were incubated with biotinylated detection antibodies and streptavidin-horse radish peroxidase conjugate (Sigma Aldrich, USA). TMB substrate (BD, USA) was used to visualize antibody binding, reaction was stopped using 1N hydrochloric acid, and measured at 450 nm.

**Endotoxin analysis**
The filters were analysed by a quantitative kinetic chromogenic Limulus Amoebocyte Lysate assay (Douwes et al., 1995) and results are expressed in EU m⁻³ (EU = endotoxin units, 10 EU = 1 ng). Analyses were performed at the National Institute of Occupational Health in Norway.

**NAGase analysis**
Measurement of NAGase activity was performed on bioaerosol samples from the edible crab industry. NAGase activity was quantified by adding 4-methylumbelliferyl N-acetyl-B-D-glucosaminide (the MUF-substrate, Sigma, USA) to Tris-maleate buffer (pH 5.0) (Frankel et al., 2012). Aerosol samples were suspended by vortex mixing followed by incubation. The enzymatic reaction was stopped and the supernatant was added to Tris buffer 2.5 M. The solution was added to a black microtiter plate and fluorescence was detected at 446 nm and excitation at 377 nm by a fluorescence spectrometer. NAGase activity was calculated by comparing sample fluorescence with that of a standard curve containing 4-MU (0-7095 pmol ml⁻¹). Analyses were performed at the National Research Centre for the Working Environment in Denmark.

**Trypsin analysis**
Protease activity in filter extracts was analysed by zymography. Five µl sample extracts were applied on zymographic gels (Novex® no.EC61752, ThermoFisher Scientific) containing a standard curve (0.014–0.228 mU ml⁻¹) prepared by dilution of a porcine trypsin stock solution with known enzyme activity. Enzyme activity of the stock solution of porcine trypsin was determined by a serine protease assay where the hydrolysation of a chromogenic substrate (DL-BAPNA, Sigma-Aldrich) was measured spectrophotometrically by the increase in absorbance at 405 nm at room temperature for 10 min. Protease activity was calculated by: Unit = dA/dt × 1/ (ε*optical path length) * 10⁶ * V_final, where dA/dt = rate of absorbance change and ε = extinction coefficient. In our system, ε was 8800 M⁻¹ cm⁻¹, optical path length 0.709 cm and V_final 250 ul. Trypsin standards and aliquots of filter sample extracts were mixed with loading buffer (Novex®, ThermoFisher Scientific) and subjected to electrophoresis at 20 mA gel⁻¹ for 2 h. Thereafter, the gel was washed with washing buffer followed by overnight incubation in developing buffer at 37°C (Novex®, ThermoFisher Scientific). The gels were stained in 0.2% Coomassie Brilliant Blue R-250 Dye and the activity of gelatin degrading proteases detected as clear zones against the undigested, stained background. The intensity of bands of porcine trypsin (23 kDa) and corresponding size bands in filter extracts, were quantified using UVP Vision Works LS Image Acquisition and Analysis (UVP, LLC, USA) with I-max (point of maximal intensity) as quantification parameter. The gelatine-degrading activity was abolished by introduction of the serine protease inhibitor aprotinin. Together with the band size and the confirmed trypsin activity in king crab (Rudenskaya et al., 2000) and liquid samples from king crab industry (own unpublished data), this strongly suggests that the protease activity in this region is due to trypsin.

**Statistical analyses**
The exposure data showed a lognormal distribution so the natural logarithms of the exposure measurements were used. Exposure data are presented as median, geometric mean (GM) and range. Endotoxin samples below detection limit (1 EU m⁻³) were set to half the value of the detection limit when incorporated in statistical analyses. Single variables were compared using independent-samples t test, ANOVAs for more than two groups. A correlation matrix using Pearson’s correlation coefficient was computed to measure any linear relationship between bioaerosol components. Due to a low precision of the zymographic analysis of trypsin, the results are presented using a semi-quantitative scale based on the 25 and 75 percentile of positive results: Low ≤ 25 percentile; 25 percentile > medium ≤ 75 percentile; high > 75 percentile. Mann–Whitney U test was used for comparing the semi quantitative results.
The statistical analyses were done using the IBM SPSS software package, version 22. \( P < 0.05 \) were considered statistically significant.

**RESULTS**

In the edible crab industry, each worker was handling either raw crab or cooked crab and the results are therefore divided into these two groups. In the king crab plants however, the raw and cooked crab areas were only partly separated, and some workers performed less stationary work during processing such as maintenance, cleaning and truck driving (Fig. 1). Thus, some workers were exposed to bioaerosols from both raw and cooked king crab. The results are therefore divided into; raw crab, cooked crab, or overlapping work tasks (both raw and cooked crab) during the shift.

The difference in exposure levels between the different work areas in king crab plants was borderline significant \((P = 0.06)\), with highest levels in raw processing (Table 1). Exposure levels to protein were not significantly different, but as with tropomyosin, the exposure levels seemed to be highest in raw crab processing.

For edible crab (Table 2), the mean tropomyosin exposure level for workers processing raw crab was significantly lower than for those processing cooked crab. Protein levels were not significantly different. Exposure to endotoxin and NAGase was highest when processing raw crab, but the differences in NAGase levels were not significant. However, endotoxin levels during processing raw crab were significantly higher than levels found during processing cooked edible crab.

Trypsin activity was measured in 36 samples, whereof 23 were positive (Table 3). In the present data set, the 25\(^{th}\) percentile was 0.16 mU m\(^{-3}\) and the 75\(^{th}\) 0.72 mU m\(^{-3}\). In the edible crab industry, there was no trypsin activity in the air samples collected from workers handling cooked crab. In the king crab industry, the levels of activity were significantly higher when processing raw crab compared to cooked crab.

When comparing exposure levels for workers handling cooked king crab and edible crab, there were significantly higher levels of both protein and allergen in edible crab processing compared to king crab (Figs 2A,B).

Table 4 shows that levels of tropomyosin and total protein significantly differ between king crab plants. Plant A was the only plant where endotoxin samples were below detection level. Plant A also had the lowest exposure levels to tropomyosin and protein of the king crab processing plants.

Using Pearson’s correlation, a significant correlation as found between protein and tropomyosin levels in both king crab \((r = 0.58, 43\text{ pairs})\) and edible crab \((r = 0.42, 24\text{ pairs})\) separately, as well as in all samples combined \((r = 0.59, 67\text{ pairs})\). A significant correlation was found between protein and endotoxin when all samples were included \((r = 0.53, 15 \text{ pairs})\), but not when king crab and edible crab were analysed separately. No correlation was found between the other components.

**DISCUSSION**

In this study, we measured total proteins, tropomyosin, endotoxin, trypsin and NAGase in bioaerosols collected in workers’ breathing zone during processing of king crab and edible crab. Endotoxin and trypsin levels were highest in king crab processing, while total protein and tropomyosin levels were highest when processing edible crab. Processing cooked crab generated higher concentrations of tropomyosin than processing raw crab (Fig. 2). However, enzyme activity was higher in bioaerosols collected in raw crab processing than in cooked (Table 3). Exposure levels varied within raw and cooked processing respectively, but also varied between the different king crab plants.

Attempts to compare and evaluate bioaerosol exposure levels are hampered by the lack of occupational exposure limits and standardized, reproducible methods for collecting and analysing the samples (Douwes et al., 1995; Eduard et al., 2012). The levels of exposure to total protein and allergens such as tropomyosin have been measured to some extent in other studies in the seafood industry. Allergen exposure during crab processing has been studied (Griffin, 1994; Malo et al., 1997; Weytjens et al., 1999; Beaudet et al., 2002; Gill et al., 2009; Abdel Rahman et al., 2012) and showed that exposure levels vary within work areas and exposure groups. Comparison of the total protein levels in our study and those in previous reports from seafood industry shows that the range of exposure in our plants (0.3–97 µg m\(^{-3}\)) are wider than the range reported in fish processing plants such as the plants studied by Jeebhay et al. (2005) in South Africa (Limit of detection: 11.50 µg m\(^{-3}\)). Total protein exposure levels ranging from 0.76 to 13 µg m\(^{-3}\) has been found in the...
Table 1. Exposure levels (median, geometric mean (GM), range = min and max measurements) of bioaerosol components in king crab workers’ breathing zone when processing either raw crab, cooked crab or performing work tasks overlapping both processing areas

<table>
<thead>
<tr>
<th>Processing area</th>
<th>Bioaerosol levels in king crab processing</th>
<th>Overlapping work tasks</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bioaerosol component</td>
<td>Samples (n)</td>
<td>Median</td>
</tr>
<tr>
<td>Tropomyosin (ng m⁻³)</td>
<td>27</td>
<td>1.6</td>
<td>2.3</td>
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<tr>
<td>Protein (µg m⁻³)</td>
<td>21</td>
<td>3.9</td>
<td>5.1</td>
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<tr>
<td>Endotoxin (EU m⁻³)</td>
<td>5</td>
<td>630</td>
<td>110</td>
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</table>

P values of ANOVA of processing raw crab, cooked crab or performing work tasks overlapping both areas.

Table 2. Exposure levels (median, geometric mean (GM), range = min and max measurements) of bioaerosol components in edible crab workers’ breathing zone when processing raw or cooked crab

<table>
<thead>
<tr>
<th>Processing area</th>
<th>Bioaerosol levels in edible crab processing</th>
<th>P values</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Bioaerosol component</td>
<td>Samples (n)</td>
</tr>
<tr>
<td>Tropomyosin (ng m⁻³)</td>
<td>10</td>
<td>20.1</td>
</tr>
<tr>
<td>Protein (µg m⁻³)</td>
<td>10</td>
<td>11.6</td>
</tr>
<tr>
<td>Endotoxin (EU m⁻³)</td>
<td>5</td>
<td>51</td>
</tr>
<tr>
<td>NAGase (pmol 4-MU m⁻³)</td>
<td>4</td>
<td>1107</td>
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</table>

P values of t tests comparing levels when processing raw and cooked crab.
Table 3. Trypsin activity from king crab and edible crab plants: filter samples were collected from workers’ breathing zone

<table>
<thead>
<tr>
<th>Exposure level</th>
<th>Trypsin activity(^a) in personal samples (number of samples within exposure category)</th>
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<th>P values</th>
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<tbody>
<tr>
<td></td>
<td>King crab</td>
<td>Zero</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>Zero</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
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<td>Production area</td>
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<td>4</td>
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<td>4</td>
<td>6</td>
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<td>0</td>
<td>4</td>
<td>0</td>
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<td>0.836</td>
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<td>Cooked</td>
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<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
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<td></td>
<td>0.282</td>
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<td>P value raw versus cooked</td>
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<td>C</td>
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<td>P value between plants</td>
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\(^a\)Trypsin activity is presented in a semi-quantitative scale based on the 25\(^{th}\) percentile (0.16 mU m\(^{-3}\)) and 75\(^{th}\) percentile (0.72 mU m\(^{-3}\)) of positive samples: Low ≤ 25\(^{th}\) percentile; 25\(^{th}\) percentile > medium ≤ 75\(^{th}\) percentile; high > 75\(^{th}\) percentile.

The table presents P values for Mann–Whitney U tests comparing processing areas (raw versus cooked), crab type (king crab versus edible crab), and king crab plants (plant A versus B and C).
Norwegian salmon industry (Shiryaeva et al., 2013) where an exposure-response relationship was found between protein exposure and self-reported respiratory symptoms and lung function test outcomes. These levels are also lower than the levels found in our study (0.3–97.5 µg m⁻³). Several studies have found a high prevalence and incidence of allergy and asthma attributed to bioaerosol exposure (Cartier et al., 2004; Neis, 2004; Howse et al., 2006; Gautrin et al., 2010; Jeebhay, 2011; Bønløkke et al., 2012). However, the levels of bioaerosols and the specific components they contain are important factors for risk assessment. Future studies need to address the question of dose-response and threshold levels in work environments. Because of unpredictable work schedules in this industry, the average exposure over shifts and seasons are difficult to predict. They did not have production every day, and shifts could vary between 2 and 12 h in length (normal shift was 8 h). This will affect the average level of bioaerosols inhaled by the workers over time. However, we found that workers were exposed to high levels of possible sensitizers and proinflammatory agents such as tropomyosin, trypsin, and endotoxins (Tables 1–3). In occupational settings, workers are not exposed to a single component, and simultaneous exposure to several components could potentially alter the effect on workers’ health. Combined effects of bioaerosol components have been poorly investigated. Bhagwat et al. (2015) showed a synergistic effect of endotoxin and seafood proteases, augmenting inflammatory cytokines in an in vitro respiratory cell model.

Inhalation exposure of allergens and subsequent atopic sensitization and reactivity leading to respiratory problems is a highly prevalent problem in the seafood industry affecting a considerable part of the workforce (Beaudet et al., 2002; Cartier et al., 2004; Howse et al., 2006; Gautrin et al., 2010; Bønløkke et al., 2012). Tropomyosin is the major
cross-reactive, heat stable, and most abundant allergen among crustaceans although several other allergenic proteins have been found (Gill et al., 2009; Abdel Rahman et al., 2012) and was therefore used as a biomarker to assess inhalation allergen exposure.

The sensitive ELISA method used to measure tropomyosin (Malo et al., 1997; Kamath et al., 2014) has no unspecific binding to other proteins and no loss of binding due to protein modification procedures that may have influenced the levels reported in other studies (Griffin, 1994; Lopata et al., 2005; Lopata and Jeebhay, 2013). Previous studies have also used the highly sensitive mass spectrometric based strategies to quantify tropomyosin and arginine kinase (Abdel Rahman et al., 2010; Abdel Rahman et al., 2011; Rosmilah et al., 2012; Abdel Rahman et al., 2012). The immunoassay used in this study had a limit of quantification of 100 picograms m\(^{-3}\) of sampled air. Since tropomyosin is heat-stable, both raw and cooked crab are possible sources for aerosolized tropomyosin (Jeebhay et al., 2001; Lopata and Jeebhay, 2013). Antibody reactivity of crustacean tropomyosin can also increase after heating, a possible result of protein denaturation and exposure of new epitopes, aggregation and chemical modifications (Kamath et al., 2013; Abramovitch et al., 2013). A study involving snow crab processing found more aerosolized allergen when workers were handling cooked crab than raw (Swanson et al., 2004) which is in line with the results of the present study.

Levels of tropomyosin were significantly higher in the edible crab plant compared to the king crab plants, which may be a result of the way the crabs are handled during processing. Both use high speed rotating cleaning brushes and have poor shielding of workers from the bioaerosols produced. However, after the king crab is cooked, the clusters are gently packed, glazed (sprayed with water), and frozen with minimal manual handling and no further processing. During edible crab processing most of the handling happens after cooking; sorting, mincing shell to extract all muscle, and packing muscle back into the shell before packaging—tasks that produce bioaerosols. The bioaerosol exposure in king crab plants were not significantly different between raw and cooked processing, apart from trypsin activity, whereas the levels at the edible crab plant were. A reason for this difference may be that workers in the king crab industry were not at a single workstation throughout the shift. Most of the

<table>
<thead>
<tr>
<th>Bioaerosol component</th>
<th>Samples Median GM Range</th>
<th>Samples Median GM Range</th>
<th>Samples Median GM Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropomyosin (ng m(^{-3}))</td>
<td>28 1.7 1.8 0.6–3.8</td>
<td>19 2.0 1.9 0.4–5.1</td>
<td>20 2.6 2.0 0.3–4.4</td>
</tr>
<tr>
<td>Protein (µg m(^{-3}))</td>
<td>20 2.6 2.0 0.3–4.4</td>
<td>16 3.9 3.8 0.4–26.6</td>
<td>12 5.6 3.3 0.1–75.9</td>
</tr>
</tbody>
</table>

*Table 4. Exposure levels (median, geometric mean (GM), Range = min and max measurements) of bioaerosol components in king crab workers' breathing zone at the three different king crab plants (A, B, and C).*

*P values of ANOVA comparing levels at the three plants.*
Handling involved raw king crab, and raw crab was also the main source of bioaerosol production. In the edible crab industry, workers were stationary throughout the shift, which may result in greater bioaerosol accumulation as workers may be exposed to bioaerosols from their own workstation, as well as those produced by nearby workers if the ventilation conditions or local exhaust ventilation is not optimal. These differences in how the crabs are processed may partly explain the differences in exposure levels between industries as well as the differences found between handling raw and cooked crab within each industry.

Endotoxin levels in the edible crab plant were lower for workers handling cooked crab than raw crab. Endotoxins are part of Gram negative bacterial cell walls. These may be abundant on the outer shell of the crabs as part of their environment. Levels are therefore expected to be highest when the shell of the crab is cleaned or removed which happens during raw processing. Endotoxin levels of 1350 EU m$^{-3}$ have been reported in herring industry (Bang et al., 2005). Endotoxins are suggested to be a cause of occupational respiratory symptoms in occupational settings including among seafood processing workers (Sherson et al., 1989; Sigsgaard and Schlunssen, 2004; Madsen et al., 2015). A 'no adverse health effect' of 90 EU m$^{-3}$ has been suggested (The Nordic Expert Group for Criteria Documentation of Health Risk from Chemicals, 144. Endotoxins 2011), a level exceeded in two of eight endotoxin samples in the edible crab plant, and in four of seven samples in the king crab plants.

Enzyme activity was significantly lower when handling cooked king crab than raw, and there was no enzyme activity in samples taken during cooked edible crab processing. A previous study found that there is trypsin and trypsin-like enzyme activity in extracted tissue samples from crustaceans (Sun and Lopata, 2010), but there are no previous publications that have quantified the level of airborne trypsin activity during seafood processing. Trypsin is a common serine protease enzyme and inhaled by workers may have an immunological or non-immunological effect on the lungs (Baur, 2005; Sun and Lopata, 2010; Florsheim et al., 2015; Madsen et al., 2015). Optimal conditions for several crustacean trypsins are around 40°C and pH6, which is close to the physiological conditions found in human lungs (Sun and Lopata, 2010). However, studies have found that heating the enzyme is enough to inactivate the enzyme which would remove the proteolytic activity from enzymes being inhaled by workers processing cooked crab (Zhang et al., 1999; Sun and Lopata, 2010; Larsen et al., 2011; Bhagwat et al., 2014).

Because chitin is a major component of crustacean shell (Espie and Roff, 1995) and NAGase is a widely distributed enzyme that digests chitin (Chen et al., 2011) we would expect NAGase to be present in the processing plants. Our samples were collected in autumn/winter and the levels were higher than levels found in normal Danish homes at that time of year (Frankel et al., 2012) so we expect the crab processing to be a contributor to the NAGase levels. There is a growing body of literature (Muzzarelli, 2010) suggesting that NAGase may have a negative impact on health by eliciting an immunological response (Allermann et al., 2006). Exposure to enzymes in occupational settings has been linked to sensitization and asthma (Green and Beezhold, 2011) as well as ODTS (Madsen et al., 2015).

We wished to investigate if there was a correlation between the components to explore the possibility of performing simple rounds of measurement where only one or a few components are measured. It would simplify exposure measurements in epidemiological studies. However, correlations such as these were not found to such a degree that we feel it is possible to replace measurements of components with other measurements.

The work task performed by each individual is not necessarily the most important factor for evaluating exposure levels. Jeebhay et al. (2005) compared similar exposure groups in two fish plants and found that the department where the subject worked explained most of the variability in exposure levels. In our study, when comparing the king crab plants (Table 4), we also find large differences in the levels of exposure at different plants. This suggests, as did studies on snow crab workers in Canada (Neis, 2004; Abdel Rahman et al., 2012), that there may be important factors to look at in addition to work tasks and whether the workers are handling raw or cooked crab. It is necessary to look at a complete 'plant effect', the sum effect of the layout of the processing line, ventilation, equipment producing bioaerosols, and the technique of each individual worker, in addition to other unmeasured covariates, when evaluating the plant. Plant A had a higher level of technical equipment compared to plants B and...
C. Plant A was also the only king crab factory not altering between crab and fish processing, thus the equipment in this plant was permanently placed unlike plant B and C. Plant A also differed by complete separation between raw and cooked processing areas by a solid wall (Fig. 1A) that prevented cooking fumes dispersing through the plant. Plant B had a separate enclosed room for the cooking vats (Fig. 1B). However, the door to the room was opened to move the crab in and out. Dispersion of cooking fumes was reduced by the door, but not removed. The ventilation system in the cooking room consisted of a large influx of air into the room, and not removal from the workers and out of the plant. Plant C had no physical barrier between the raw and cooked processing (Fig. 1C). The difference in bioaerosol levels found between plants may in part be because of these differences. This also illustrates the difficulties of transferring measurements from one plant to generalization about all crab processing plants and the importance of local variations.

In this study, several important components of bioaerosols have been identified and quantified. The presence of allergens in air samples demonstrate that it is possible for workers to be sensitized by airway exposure. For further assessment of health risks from occupational bioaerosol exposure, dose-response studies with identification of no observed adverse effect levels for individual components are important for developing occupational exposure threshold limits. Studies of combined effects of bioaerosol components are needed. Identification of specific allergens and in which part of crab processing these allergens are aerosolized would aid the priorities in implementation of preventive measures.

CONCLUSIONS

In Norwegian crab processing plants, workers are exposed to bioaerosols containing endotoxins and proteins including tropomyosin, NAGase, and trypsin. Higher levels of total protein and tropomyosin were found during processing in the edible crab industry than in the king crab industry, particularly in the cooking area. A significant difference was found between king crab plants in terms of protein, tropomyosin, and trypsin levels, suggesting a ‘plant effect’.

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