Exposure to bioaerosols during fish processing on board Norwegian fishing trawlers

Heidelberg CT[1,2], Bang B[1,2], Thommassen MR[1], Kamath SD[3], Reuthers T[3], Lopata AL[3],

Madsen AM[4], Sandsund M[5], Aasmoe L[1,2]

[1] University Hospital North Norway, Department of Occupational and Environmental
   Medicine, Tromsø, Norway
[2] UiT The Arctic University of Norway, Department of Medical Biology, Tromsø, Norway
[3] Australian Institute of Tropical Health and Medicine, College of Public Health, Medical and
Veterinary Sciences, Townsville, Australia
[4] National Research Centre for the Working Environment, Copenhagen, Denmark
[5] SINTEF Digital, Department of Health Research, Professor Brochs gt. 2, 7030 Trondheim

Corresponding author:

Aasmoe L: lisbeth.aasmoe@unn.no

Bang B: Berit.Elisabeth.Bang@unn.no

Abstract

Introduction: The main objective was to gain more knowledge on exposure to bioaerosols in the processing area onboard fishing trawlers.

Methods: Exposure samples were collected from 64 fishermens breathing zone and from stationary sampling stations on board five deep-sea fishing trawlers (1–5).

Trawler 2, 3 and 4 were old ships, not originally built for on board processing of the catch. Trawler 1 and 5 were relatively new and built to accommodate processing machineries. On trawler 1-4 round fish was produced; the head and entrails were removed before the fishes were frozen in blocks. Trawler 5 had the most extensive processing, producing fish fillets. Samples were analyzed for total protein, trypsin activity, parvalbumin and endotoxin. One side ANOVA and Kruskal-Wallis H Test was used to compare levels of exposure on the different trawlers.

Results: Personal exposure to total protein were higher on the three oldest trawlers (2, 3 and 4) compared to the two new trawlers (1 and 5). Highest activity of trypsin was detected on the four trawlers producing round fish (1-4). Parvalbumin was detected in 58% of samples from the fillet-trawler (5) compared to 13% of samples from the four trawlers producing round fish. The highest level of endotoxin was detected when using high-pressure water during cleaning machines and floors in the processing area.

Conclusion: Fishermen in the processing area on board Norwegian trawlers are exposed to airborne bioaerosols as proteins, trypsin, fish allergen parvalbumin and endotoxin. Levels varied between trawlers and type of production.

Keywords

exposure to bioaerosols, fishing trawlers, fish processing, proteins, trypsin, parvalbumin, endotoxin

Introduction

Fishermen are exposed to potentially harmful working conditions. Due to a high number of occupational deaths and reported accidents in fisheries, considerable research emphasis has been on safety, accidents and documenting the number of fatalities and injuries (Windle, 2008; Aasjord, 2012; Frantzeskou et al., 2014; Jensen, 2014). There is, however, a lack of knowledge on workers exposures to bioaerosols during seafood processing on board fishing trawlers. Bioaerosols are airborne particles of biological origin. Several studies in onshore seafood industry plants have shown that production workers are exposed to bioaerosols generated by activities such as mechanical processing of the fish or cleaning by water jets directed towards biological materials (Bang et al., 2005; Jebhay et al., 2004; Jeebhay et al., 2005; Dahlman-Høglund et al., 2013; Shiryaeva et al., 2014; Thomassen et al., 2016). Exposure to bioaerosols during seafood processing may increase the risk of developing upper and lower respiratory symptoms, asthma and allergy (Jeebhay et al., 2010; Bang et al., 2005; Douglas et al., 1995; Shiryaeva et al., 2014; Dahlman-Höglund et al., 2012; Thomassen et al., 2016). Extensive use of water as well as machineries, generate squirts, airborne droplets and particulate matter that may be inhaled by the workers. These aerosols may contain bioactive agents, including enzymes, allergens and endotoxins. Confined spaces are common on vessels, and the processing areas on board are characterized by non-shielded machines and processing lines. Previous research has revealed higher levels of crab allergens during processing the crab on board crab fishing vessels compared to a land-based crab plant (Baudet, 2002; Weytjens, 1999). It has also been shown that Russian trawler fishermen were more likely to have asthma, respiratory symptoms and impaired lung function compared to Russian

merchant seafarers, suggesting that the work environment on board trawlers contains risk factors for respiratory symptoms (Shiryaeva at al., 2011).

Total protein levels give an indication of the total load of proteins workers are exposed to, and may be used as an indicator of bioaerosol exposure (Berg et al., 2003). When seafood is processed and bioaerosols are released into the air, they very likely include proteins. The proteins of respirable range will enter the airways and may affect the respiratory system causing e.g. rhinitis and occupational asthma (Jeebhay et al., 2001, Jeebhay and Cartier, 2010, Jeebhay 2011). Since measuring total protein fraction is a comparatively quick and easy way to examine the load of organic components in bioaerosols, it may serve as an indicator of occupational exposure to biological components. However, studies have found that this is not a good indicator for specific components such as allergens (Thomassen at al, 2016, Jeebhay et al., 2005, Heederik et al., 1999), nor does it measure bioaerosols that are not protein based, as endotoxins. Endotoxin is a lipopolysaccharide component of the cell-wall of Gram-negative bacteria, and inhalation may induce an inflammatory response in the respiratory system (Eduard et al., 2009). Trypsin is a common digestive enzyme, catalyzing protein hydrolysis in vertebrates and invertebrates. Exposure to trypsin is linked to innate inflammatory responses in cell models and suspected to play a role in allergy, including seafood allergies (Sun and Lopata, 2010; Baur 2005; Larsen et al., 2008). In a previous study, airborne trypsin was detected in processing areas in the crab industry (Thomassen et al., 2016). Parvalbumin, a muscle protein, is the major allergen in bony fish and has been demonstrated to be one of the airborne allergens responsible for allergic respiratory disease in seafood-processing workers (Lopata and Jeebhay, 2013; Beale at al., 2009).

Exposure to bioaerosols in the work environment in fishing vessels is sparsely described. Based on previous investigations in fish processing industry on shore, and the sparse existing

data from fishing vessels, we hypothesize that fishermen working in the processing area on board trawlers are exposed to aerosols containing biological active compounds as enzymes, allergens and endotoxins, and that the design of the processing area is important with regard to the exposure levels.

Objectives

The main objective was to gain more knowledge on exposure to bioaerosols in the processing area on trawlers through quantifying total protein, trypsin, endotoxin and parvalbumin levels, collected through personal and stationary aerosol sampling, and to compare the exposure levels on different trawlers.

Materials and Methods

Study population

The trawlers were recruited in collaboration with the ship owners. Important criteria to be included in this study was that the trawlers were fishing white fish in the Barents and Norwegian Sea, processed the fish on board in the factory area, and had spare cabins to accommodate the researchers. Based on these criteria, five trawlers were included in this study.

Fishermen working in the processing area on board, voluntarily carried bioaerosol sampling equipment during their work shifts. In total, 64 fishermen from the five trawlers participated in the study.

During sampling on board, the fishermen working in the processing area were asked to report which work tasks they performed during the shift, and also whether they cleaned machines, equipment and floors during the shift, and time spent to perform the different tasks. Written informed consent was obtained from all participants. The study was approved by the Regional Committee for Medical Research Ethics at The Arctic University of Norway, Tromsø.

Participant trawlers

Data were collected on five Norwegian trawlers in 2014 and 2015 during regular activity/fishing in the Norwegian Sea and in the Barents Sea. Four freeze-trawlers (numbered 1-4), and one fillet-trawler (number 5) were included. At the time of data-collection, the age of the trawlers 2, 3, and 4 were 15-21 years old, and trawler 1 and 5 was 3 years old. Each trip at sea lasted from 9 to18 days.

Fishermen on freeze-trawlers operates machinery that removes head and entrails of each fish. The fishes are stored for a short time in tanks to bleed out, before they were sorted and cleaned. The fishes were distributed into vertical plate freezers and are frozen into blocks of 25 or 50 kg. The frozen blocks were removed from the plate freezer, and placed at the cold storage on a lower deck until they were offloaded onshore. The fillet-trawler processed the fish further into fillets after decapitation and degutting. The fillets were manually checked for bones and trimmed, sorted after weight and packed, frozen, and stored in the freezer hold. Trawler 1 and 5 produced fish-meal in a separate room.

Schedule

Seafood processing on board is continuous work, divided into four shifts, each lasting for six hours. Fishermen followed a rotation of six hours on and six hours off. A work period lasted for 4-6 weeks, including work on the trawler deck, fish processing in the factory area below deck, moving to new fishing areas, and delivering the catch on shore when the freezer hold is fully loaded, sometimes several times during the work period. During a shift, each fisherman may work both on the trawler deck and in processing area, or only one of these locations.

Exposure sampling

Exposure sampling was performed during processing of fish with stationary samplers placed in the factory area or personal samplers carried by the fishermen and connected to filters in their breathing zone. SKC Sidekick (SKC Ltd., Dorset, UK) sampling pumps were connected to samplers containing filters specific for each exposure agent. The flow rate for each pump was approximately 3 l/min. The flow rate was controlled before and after collection using Bios Defender 520 (SKC Ltd., Dorset, UK). If the flow at the end of the sampling period had deviated with more than 10% from the start of the sampling, the samples were discarded.

Due to confined spaces in the processing area and safety considerations, fishermen carried only one sampling pump connected to samplers. At break times, and when workers were outside on the deck, the pumps were stored away from the processing area, but kept sampling to represent the mean exposure of the fishermen during a shift, including time away from the processing area. Also due to safety considerations and confined spaces the samplers for stationary measurements were placed randomly at locations in the processing area on each trawler.

Samples for proteins, trypsin and allergen were collected using SurealSeal Air monitoring cassettes (37 mm, 3-pc, styrene SKC Ltd. UK) on polytetrafluoroethylene (PTFE/Teflon) filters with polypropylene support (37 mm, 1.0 mm SKC Ltd. UK). The cassettes were stored at -20°C on board the trawlers, and after at -70°C after arrival to the laboratory until extraction and analysis. Samples for endotoxin were collected using preloaded cassettes with glass fiber filters, type AE, 37 mm, 3 piece, pre-banded (SKC 225-706). The cassettes were stored at -20°C until extraction.

262 personal samples from 64 fishermen and 108 stationary samples were sampled and analysed for total protein. The sampling time for personal and stationary samples was approximately 340 and 600 minutes respectively. Trypsin was analysed in 252 of 262 of the personal protein samples. In addition, 72 stationary samples for parvalbumin analysis and 67 stationary samples for endotoxin analysis were sampled.

Total protein analysis

The protein filters were extracted in 1000 µl PBS with 0.5% Tween20. Manual QuantiPro BCA Assay Kit (Sigma-Aldrich, St. Louis, USA) was used to determine the total amount of proteins in filter extracts in the samples by colorimetric reading in a spectrophotometer at 560nm. Cut-off for quantification of total protein levels in extracts were 0.5 µg/ml.

Trypsin analysis

Trypsin activity in filter extracts was analyzed by zymography, using sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 0.1% gelatin as substrate added in the polyacrylamide gel (Bang et al 2018). Due to low precision in the early assay set up, the results are presented using a semi-quantitative scale based on the 25th and 75th percentile of positive results: Low $\leq$ 25th percentile; 25th percentile > medium percentile $\leq$ 75th percentile; high > 75th percentile. Low level $\leq$25th percentile (~0.0014 mU/m^3) of positive samples. High level $\geq$ 75th percentile (~0.0095 mU/m^3) of positive samples. One Unit is defined as dA/dt x $1/(\varepsilon$ x optical path length) x 10^6 x V_{final}, where dA/dt is the rate of absorbance change, and ε is the extinction coefficient 8800 M^{-1}cm^{-1}. Analysis of protein and trypsin were performed at the Department of Occupational and Environmental Medicine, University Hospital North Norway, Tromsø.

Allergen analysis

The bioorganic matter from the air-filters were extracted using 1 mL of extraction buffer (100 mM ammonium Bicarbonate, 0.05% Tween10, and 2 nM sodium azide under agitation for 3h at 28ºC using a previously established protocol (Kamath et al 2014). For quantification of the major fish allergen, parvalbumin, 60 ul of the filter extract were loaded on a nitrocellulose membrane (Biorad, USA) using the PR 600 Slot Blot Filtration Manifolds (Amersham Biosciences), followed by air drying for 10 min. Membranes were then blocked in 5% skimmed

milk (in PBS) for one hour, followed by incubation with an anti-parvalbumin antibody cocktail (antibody generated against parvalbumin from barrmundi (*Lates calcarifer*), basa (*Pangasius hypophthalmus*), Atlantic salmon (*Salmo salar*), and pilchard (*Sardinops sagax*)); diluted 1:1,000 in PBS with 1% skimmed milk and 0.1% Tween20 (PBS-T) for 20 minutes (Sharp et al 2015). After washing with PBS-T the membranes were incubated with the secondary (anti-rabbit IgG) antibody labeled with horseradish peroxidase (HRP) for 20 minutes and washed as before. Detection of parvalbumin was visualized using the enhanced chemiluminescent (ECL) assay as described previously (Kamath et al., 2013). For the quantification of parvalbumin in the filter extracts, a standard curve was generated by serial dilution of purified parvalbumin from Atlantic cod (*Gadus morhua*) dotted on the membrane as described above. The limit of detection was achieved up to 0.8 µg/filter which corresponds to approximately 0.4 µg/m^3 of the allergen parvalbumin in the samples. Analyses were performed by co-operators at James Cook University, Australia

Endotoxin analysis

The glass fibre filters were extracted in 4.0 ml pyrogen-free water with 0.05% Tween 20 by orbital shaking (300 rpm) at room temperature for 60 min and centrifuging (1000 g) for 15 min. The filters were analysed for endotoxin content by a quantitative kinetic chromogenic Limulus Amoebocyte Lysate assay (Kinetic-QCL endotoxin kit, Lonza, Walkersville, MA, USA), and results were expressed in EU m^{-3} (EU = endotoxin units, 10 EU = 1 ng) (Madsen et al 2014). Analyses were performed at the National Research Centre for the Working Environment, Copenhagen, Denmark.

Statistical analysis

The exposure data showed a lognormal distribution so the logarithms of the exposure measurements were used for analyses assuming a normal distribution. Exposure data are presented as arithmetic mean and median with standard deviations. Single variables were compared using one-way ANOVA for comparing the five trawlers. For analysis of trypsin, the results are presented using a semi-quantitative scale based on the 25th and 75th percentile of positive results. Low ≤ 25th percentile; 25th percentile > medium ≤ 75th percentile; high >75th percentile. Kruskal-Wallis H Test was used for comparing the semi quantitative results. The statistical analyses were done using the IBM SPSS software package, version 25 where $p < 0.05$ were considered statistically significant.

Results

Four freeze-trawlers producing round frozen fish and one fillet-trawler producing fish fillets were included in the study. The catch consisted mainly of a mixture of Atlantic cod, Haddock, Pollack and Deepwater redfish. Exposure samples were collected in the processing area on board trawlers during fish processing, and samples were analysed for total protein, trypsin, endotoxin and the fish allergen parvalbumin.

Personal exposure levels to total protein differed significantly between the trawlers. Environmental levels of total protein measured at stationary locations also significantly differed between the trawlers. These data are shown in table 1 and 2. Personal exposure levels to total protein from fish-meal production on trawlers 1 and 5, showed exposure levels 3.1-12.7 $\mu g/m^3$ (n=6) (data not shown). Environmental levels of total protein from fish-meal production on these trawlers showed exposure levels 0-9.1 $\mu g/m^3$ (n=6) (data not shown).

Based on a semi-quantitative scale, high levels (> 75 th percentile) of trypsin were detected in 34.7% (mean), (range 11.8-52%), of samples from the four freeze-trawlers (trawler 1-4) compared to 2.7% of samples from the fillet trawler (trawler 5), table 3. Levels of trypsin from fish-meal production on trawler 1 and 5 indicated exposure levels 1-2 (n=6) (data not shown).

Exposure levels of endotoxin, shown in table 4, also differed significantly between trawlers. The highest level of endotoxin were detected in the processing area on the fillet-trawler (n=9, range 0.5-483.6 EU/m^3) compared to the freeze-trawlers (n=58, range 1.5-111.6 EU/m^3). Endotoxin levels from fish-meal production on trawlers 1 and 5 indicated exposure levels < 16 EU/m^3 (n=6) (data not shown).

Parvalbumin was detected in 58% of the samples on the fillet trawler in contrast to 13% of the samples on the 4 freeze-trawlers as shown in table 5. Parvalbumin was not detected in samples from fish-meal production.

Discussion

To our knowledge, this is the first study reporting exposure to bioaerosols in the fish processing area on board fishing trawlers. In this study, total protein, trypsin, endotoxin and parvalbumin were analyzed in bioaerosol samples collected both from fishermens breathing zone and from stationary sampling stations.

As in land based seafood industry there has been technological developments in the processing of fish on board fishing trawlers, which has led to less manual work and more mechanical processes. Older trawlers were originally designed and built for manual handling and manual processing of the fish. In the processing area of the three oldest trawlers (2-4), space was maximally utilized with machines, and was characterized by limited space for movement and low ceilings in contrast to the two new trawlers (1 and 5) with higher ceilings and more space between the equipment. The catch consisted mainly of a mixture of Atlantic cod, Haddock, Pollack and Deepwater redfish on all five trawlers. The different species may express different characteristics with regard to e.g. allergens and other proteins. However, we cannot conclude on the effects of different species in this study. The total protein levels in the processing area on the trawlers (LOD-83 ug/m^3) were higher compared to studies performed in land based seafood industries; Norwegian salmon industry: 1-13 µg/m^3 (Shiryaeva et al., 2014), Norwegian crab industry: 2-48 µg/m^3 (Thomassen et al., 2016), African pilchard and anchovy industry: LOD-11.5 µg/m^3 (Jeebhay et al., 2005). Even if new trawlers are designed

and built for machineries with higher ceilings and more space, the processing areas on board trawlers are generally smaller compared to land based seafood processing plants.

Fish processing operations on board the four freeze-trawlers were in general similar. Personal exposure to total protein were higher on the three oldest trawlers (2, 3 and 4) compared to the two new trawlers (1 and 5). This was not reflected in the stationary samples and may be a result of the placement of the stationary sampling pumps. Due to safety considerations and confined spaces the samplers for stationary measurements were placed randomly at locations in the processing area on each trawler. Several factors may contribute to the higher personal exposure levels on the oldest trawlers, such as more confined spaces in the processing area, lower ceilings, and placement and efficacy of the ventilation systems. In addition, levels may be influenced by season, catch type, different processing techniques in the freeze-trawlers and fillet-trawlers, and lay-out of the processing area, as well as work load during exposure sampling. Even if the freeze-trawlers had almost identical processing lines, the observed differences might be attributable to the differences in physical layout and ventilation.

Trypsin is a serine protease located mainly in pancreatic tissue and intestines. When inhaled, proteases may have an immunological or non-immunological effect on the lungs (Sun and Lopata, 2010; Baur, 2005; Florsheim et al., 2015; Madsen et al., 2015) In a previous study in the crab industry protease activity in bioaerosol samples varied by industrial processes as well as factory-specific factors (Thomassen et al., 2016). An important work task on freeze-trawlers is to remove the entrails of the fish. Even if this work is partly automated, fishermen are in close contact with the entrails, which may explain why the highest trypsin levels were found on board freeze-trawlers.

Parvalbumin is an allergen abundant in the fish muscle. This may explain why parvalbumin was detected more often in the fillet-trawler compared to the freeze-trawlers. Parvalbumin was detected in 58% of the samples on the processing area on the fillet-trawler (7 of 12 samples) in contrast to 13% of the samples on the 4 freeze-trawlers (8 of 60 samples). This finding is in accordance with previous studies in in the processing area in herring and salmon industries. A study in the herring industry showed that workers were highly exposed to parvalbumin during handling and controlling of the herring fillets, and the lowest exposure levels in the loading and packing areas (Dahlman-Høglund et al., 2013). In the salmon industry exposure levels of parvalbumin were higher in the fillet department compared to the slaughter department (Shiryaeva at al., 2014).

Endotoxin exposure levels were highest in samples from the fillet-trawler (table 4). Several factors may contribute to this. A known risk factor for increased endotoxin levels is the use of high-pressure water in cleaning procedures (Visser et al., 2006; Madsen et al., 2013; Shiryaeva et al., 2014). In land-based seafood plants, the production areas are normally cleaned after work shifts, during the night, and often performed by external workers. On board trawlers, the cleaning of machines, floors and other equipment is done by the fishermen themselves. They normally use high-pressure water during cleaning, and the main cleaning is performed when the trawler relocates to another fishing location, or is on its way to offload frozen fish on shore.

Normal use of high-pressure cleaning in the processing area is often several times during a shift, 5-15 min each time. In contrast, on the shift with the highest measured endotoxin levels, 520 and 548 EU/m^3, the fishermen reported use of high-pressure cleaning for several hours. The high endotoxin levels may be due to accumulation and growth of microorganisms in the machines and other equipment in the processing area, that became airborne during this

prolonged cleaning period. Endotoxin exposure samples were taken as stationary samples; however, this indicates that fishermen may be exposed to high levels of endotoxin during cleaning with extensive use of high-pressure water in the processing area over a longer period of time. The measured endotoxin concentration was lower than concentration measured during high-pressure cleaning of a pig farm, and higher than the level during high-pressure cleaning at a sewage treatment plant (Madsen et al., 2013). Protein exposure levels were also examined on the specific day with high endotoxin exposure levels. However, protein exposure levels did not seem to be affected by this work process.

To our knowledge, this is the first comprehensive set of exposure data gathered from the five trawlers during fishing in the catch processing on board. The knowledge gained is highly valuable in the search to understand why trawler fishers have increased respiratory symptoms and asthma compared to merchant seamen (Shiryaeva et al,. 2011). By this the results enables demanded exposure-response modelling adding to the knowledge base necessary to suggest preventive measures and set occupational exposure limits for these workplaces in the future. The limitations of the data set were mainly due to a challenging logistics and safety considerations. The researchers needed to stay on board for a week or more at the time, often in harsh weather conditions in the Barents Sea. The type of catch and processing could not be planned in detailed beforehand, as the fish hunting is largely opportunistic in nature. The narrow conditions in the processing areas and the ship motions limited the placing of stationary exposure measurement equipment. Each fisher could only carry one personal sampling pump to reduce the risk of hooking to machineries and other equipment during sampling.

Conclusions

Fish processing workers on board deep-sea fishing vessels are exposed to proteins, trypsin, endotoxin and the major fish allergen parvalbumin. The bioaerosol levels varied significantly between vessels, and may be influenced by different processing techniques and cleaning procedures. If retrofitting is considered, great care should be taken to improve space and ventilation considered the risk of high aerosol levels suggested by this study. Older trawlers are replaced with new-built trawlers from time to time, however, there are still many of the old vessels in operation.

Conflict of interest

The authors declare that there are no conflicts of interest

References

Aasjord H. Tools for improving safety management in the Norwegian fishing fleet occupational accidents analysis period of 1998-2006. (2006) Int Marit Health; 57: 1-4.

Bang BE, Aasmoe L, Aamodt BH et al. Exposure and airway effects of seafood industry workers in northern Norway. (2005) J Occup Environ Med; 47(5): 482-92.

Bang BE, Malla N, Bhagwat SS, Aasmoe L, Winberg JO. (2018) A sensitive assay for proteases in Bioaerosol samples: Characterization and quantification of airborne proteases in salmon industry environments. Ann Work Expo Health; 62(8): 942-952.

Baur X. Enzymes as occupational end environmental respiratory sensitisers. (2005) Int Arch Occup Environ Health; 78: 279-286.

Beaudet N, Brodkin CA, Stover B et al. (2002) Crab allergen exposure aboard five crab-processing vessels. AIHA; 63: 605-9.

Beale JE,Jeebhay MF, Lopata AL. (2009) Characterization of purified parvalbumin from five fish species and nucleotide sequencing of this major allergen from Pacific pilchard, Sardinops sagax. Mol Immunol; 46(15): 2985-2983.

Berg JM, Tymoczko JL, Friend JA, Reid TM. (2003) Biochemistry 5th ed. United States of America; W.H. Freeman and Company: 41-76.

Dahlman-Höglund A, Renström A, Larsson PH et al. (2012) Salmon allergen exposure, occupational asthma, and respiratory symptoms among salmon processing workers. Am J Ind Med; 55: 624-630.

Dahlman-Høglund A, Renstrøm A, Acevedo F, Andersson E. (2013) Exposure to parvalbumin and aerosols among herring processing workers. Ann Occup Hyg; 57(8): 1020-1029.

Douglas JDM, McSharry C, Blaikie L, Morrow T, Miles S, Franklin D. (1995) Occupational asthma caused by automated salmon processing. Lancet; 737-740.

Eduard W Pearce N, Douwes J. (2009) Chronic bronchitis, COPD, and lung function in farmers, the role of biological agents. Chest; 136 (3): 716-725.

Frantzeskou E, Anastasia N, Riza E et al. (2012) Risk factors for fishermen's health and safety in Greece. Int Marit Health; 63(3): 155-61.

Frantzeskou E, Jensen OC, Linos A. (2014) Prevalence of health risk factors among fishermen – a review. Occup Med Health Aff; 2 (2): 157-161.

Heederik D, Doekes G, Nieuwenhuijsen MJ. (1999) Exposure assessment of high molecular weightsensitisers: contribution to occupational epidemiology and disease prevention. Occup Environ Med: 56(11): 735-41.

Jeebhay, MF, Robins TG, Lehrer SB, Lopata AL. (2001) Occupational seafood allergy: a review. Occup Environ Med; 58(9): 553-62

Jeebhay, MF, Robins TG, Lopata AL. (2004) World at work: fish processing workers. Occup Environ Med; 61(5): 471-4.

Jeebhay MF, Robins TG, Seixas N, Baatjies R, George DA, Rusford E, Lehrer SB, Lopata AL. (2005) Environmental exposure characterization of fish processing workers. Ann Occup Hyg; 49(5): 423-37.

Jeebhay MF, Quirce S. (2007) Occupational asthma in the developing and industrial world: a review. Int J Tuberculosis and lung disease; 11(2): 122-33.

Jeebhay MF, Cartier A. (2010) Seafood workers and respiratory disease: an update. Curr Opin Allergy Cim Immunol; 10: 104-113.

Jeebhay MF. (2011) Occupational allergy and asthma in the seafood industry – emerging issues. Occup Health Southern Africa; 17(6): 10.

Jeebhay MF, Lopata AL. (2012) Chapter 2- Occupational Allergies in Seafood-Processing Workers. In: Jeyakumar H ed: Advances in food and nutrition research. Volume 66: Academic Press; 122-33.

Jensen OC, Petursdottir G, Abrahamsen A, Lincoln J.(2014) A review of fatal accident incidence rate trends in fishing international. International Maritime Health; 65 (2): 47-52.

Kamath, SD, Abdel Rahman AM, Komoda T and Lopata AL (2013). Impact of heat processing on the detection of the major shellfish allergen tropomyosin in crustaceans and molluscs using specific monoclonal antibodies. Food Chemistry; 141 (4): 4031-4039.

Kamath, SD, Thomassen MR, Saptarshi SR, Nguyen HM, Aasmoe L, Bang BE and Lopata AL. (2014) Molecular and immunological approaches in quantifying the air-borne food allergen tropomyosin in crab processing facilities. International Journal of Hygiene and Environmental Health; 217(7): 740-750.

Larsen AK, Seternes OM, Larsen M, Aasmoe L, Bang B. (2008) Salmon trypsin stimulates the expression of interleukin-8 via protease-activated receptor-2. Toxicol Appl Pharmacol; 230: 276-282.

Lopata AL, Jeebhay MF. (2013) Airborne seafood allergens as a cause of occupational allergy and asthma. Curr Allergy Asthma Rep; 13: 21-28.

Lucas D, Lodde B, Jepsen JR, Dewitte J-D, Jegaden D. (2016) Occupational asthma in maritime Environments: an update. Int Marit Health; 3: 144-152.

Novalbos J, Nogueroles P, Soriguer M, Piniella F. (2008) Occupational health in the Andalusian fisheries sector. Occup Med; 58: 141-43.

Madsen AM, Matthiesen CB. (2013) Exposure to aerosols during high-pressure cleaning and relationship with health effects. Ann Agr Environ Med; 3:420-425.

Madsen AM, Tendal K, Frederiksen MW. (2014) Attempts to reduce exposure to fungi, β-glucan, endotoxin and dust in vegetable greenhouses and packaging units. Sci Tot Environ; 468-469: 1112-1121.

Sharp MF, Stephen JN, Kraft L, Weiss T, Kamath SD and Lopata AL. (2015) Immunological cross-reactivity between four distant parvalbumins-Impact on allergen detection and diagnostics. Molecular Immunology; 63(2): 437-448.

Sherson D, Hansen I, Sigsgaard T. (1989) Occupational related respiratory symptoms in trout-processing workers. Allergy; 44(5): 336-41.

Shiryaeva O, Aasmoe L, Straume B, Bang B. (2011) An analysis of the respiratory health status among seafarers in the Russian trawler and Merchant fleet. Am J Ind Med; 54: 971-79.

Shiryaeva O, Aasmoe L, Straume B, Olsen AH, Ovrum A, Kramvik E, Larsen M, Renstrøm A, Merritt AS, Heldal KK, Bang B. (2014) Respiratory effects of bioaerosols: Exposure-respons study among salmon-processing workers. Am J Ind Med; 57(3): 276-85.

Sun S, Lopata AL. (2010) The role of shellfish proteases in allergic diseases and inflammation. Curr Allergy Clin Imm; 3(4): 174-179.

Thomassen MR, Kamath SD, Madsen AM, Eduard W, Bang BE, Aasmoe L. (2016) Occupational exposure to bioaerosols in Norwegian crab processing plants. Ann Occup Hyg; 60(7): 781-94.

Visser MJ, Spaan S, Arts HJJM, Smit LAM, Heederick DJJ. (2006) Influence of different cleaning practices on endotoxin exposure at sewage plants. Ann Of Occupp Hyg; 1-6.

Weytjens K, Cartier A, Malo JL, Chrétien P, Essiembre F, Lehrer S, Swanson M. (1999) Aerosolized snow-crab allergens in a processing facility. Allergy; 54:892-902.

Windle MJS, Neis B, Bornstein S, Binkley M, Navarro P. (2008) Fishing occupational health and safety: A comparison of regulatory regimes and safety outcomes in six countries. Marine Policy; 31: 701-710.

Table 1: Personal total protein exposure levels in the processing area on 5 trawlers.

*Trawler nr (no, type)	Arithmetic mean ±SD μg/m³ (n)	Median μg/m³	Range min-max	Geometric mean μg/m³ (n)
1 freeze-trawler	9.7±8.5 (50)	7.9	BC-39.8	9.17 (41)
2 freeze-trawler	15.1±11.6 (46)	13.2	1.3-56.9	10.97 (46)
3 freeze-trawler	13.3±16.1 (34)	8.9	BC-83.2	7.86 (33)
4 freeze-trawler	13.7±5.8 (49)	13.5	1.0-29.0	12.23 (49)
5 fillet-trawler	5.3±4.8 (77)	4.6	BC-34.1	4.03 (75)

BC: below cut-off

* ANOVA between trawlers showed significant difference in exposure levels $p < 0.001$

Table 2: Stationary total protein exposure levels in the processing area on 5 trawlers.

*Trawler (no, type)	Arithmetic mean ±SD μg/m³ (n)	Median μg/m³	Range min-max	Geometric mean μg/m³ (n)
1 freeze-trawler	2.1±2.3 (22)	1.5	BC-10.8	2.29 (15)
2 freeze-trawler	4.9±2.3 (19)	5.1	1.1-9.0	4.17 (19)
3 freeze-trawler	10.6±14.0 (18)	5.4	BC-56.4	5.85 (18)
4 freeze-trawler	5.4±3.8 (24)	4.4	BC -11.7	4.79 (22)
5 fillet-trawler	5.3±3.7 (14)	4.7	1.3-13.2	4.08 (14)

BC: below cut-off

* ANOVA between trawlers showed significant difference in exposure levels $p < 0.001$

Table 3: Personal trypsin exposure levels in the processing area on five trawlers. Exposure levels are presented in a semi-quantitative scale based on 25th percentile and 75th percentile of positive samples.

*Trawler (no, type) (number of samples) N = 246	Zero level activity (% of samples)	Low level activity (% of samples)	Medium level activity (% of samples)	High level activity (% of samples)
1 freeze-trawler (n=47)	23.4	6.4	44.7	25.5
2 freeze-trawler (n=44)	4.5	-	54.5	40.9
3 freeze-trawler (n=34)	44.1	5.9	38.2	11.8
4 freeze-trawler (n=48)	2.1	6.3	39.6	52.1
5 fillet-trawler (n=73)	16.4	17.8	63.0	2.7

Low≤25th percentile (≤~0,0014 mU/m³) of positive samples

High>75th percentile (>~0,0095 mU/m³) of positive samples

* Kruskal-Wallis H between trawlers showed significant difference in exposure levels $p < 0.001$

Table 4: Stationary endotoxin exposure levels in the processing area on 5 trawlers

*Trawler (no, type)	Arithmetic mean ±SD EU/m³ (n)	Median EU/m³	Range min-max	Geometric mean EU/m³ (n)
1: freeze-trawler	19.5±16.4 (n=16)	14.7	2.9-67.0	14.81 (16)
2: freeze-trawler	28.5±26.7 (n=12)	16.8	6.0-94.3	20.29 (12)
3: freeze-trawler	40.2±20.7* (n=12)	35.4	16.0-82.1	35.72 (12)
4: freeze-trawler	49.7±39.2* (n=14)	33.2	7.6-111.6	34.41 (14)
5: fillet trawler	203.9±246,3 (n=7)	47.6	5.9-547.8	57.49 (7)

* ANOVA between trawlers showed significant difference in exposure levels p=0.021

Table 5: Stationary parvalbumin exposure levels (arithmetic mean, median and range,) in the processing area on four freeze-trawlers (1, 2, 3 and 4) and one fillet trawler (5).

Trawler (no, type)	Aritmetic Mean ±SD µg/m³ (n)	Median µg/m³	Range min-max
Four freeze-trawlers	1.23±0.98 (in 8 of 56)	0.76	LOD-3.50 (n=56)
One fillet-trawler	2.11±2.06 (in 7 of 9)	1.4	LOD-6.53 (n=9)

LOD: The limit of detection was approximately 0.4 µg/m³ of parvalbumin in the samples