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# Occupational exposure, respiratory health and sensitisation among crab processing workers

*A study among processors of king crab (*Paralithodes camtschaticus*) and edible crab (*Cancer pagurus*) in Norwegian land based crab processing plants*

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## Summary

Occupational asthma and allergy are health problems found in the seafood processing industry. Several factors contribute to development of respiratory health problems, including bioaerosols containing biologically active agents that are inhaled by the workers during processing. Through this work, we wished to investigate the described challenges in Norwegian crab processing plants and identify the determinants of risk to the workers' health.

The aim of this thesis was to investigate the exposure to central components in bioaerosols collected in the breathing zone of crab processing workers, and explore the workers' prevalence of respiratory symptoms, asthma and sensitisation to crustacean allergens.

This thesis describes the exposure levels of total protein, tropomyosin, trypsin and endotoxin in the breathing zone of processing workers in king crab and edible crab processing plants, in addition to NAGase in edible crab processing. This work establishes that both king crab and edible crab processing workers are exposed to bioaerosols containing these components.

When comparing king crab and edible crab processing, king crab processing results in highest levels of endotoxin while edible crab processing has the highest levels of tropomyosin and total protein. Processing procedures as well as processing plants are important determinants for exposure levels. Tropomyosin exposure are highest during cooked edible crab processing and lowest during cooked king crab processing. Trypsin activity is highest in raw processing in both king and edible crab plants. Differences in exposure levels is found between the king crab processing plants suggesting a plant effect where local differences in production, ventilation and plant layout is important for the exposure to bioaerosols.

This work also describes the increased prevalence of respiratory symptoms among crab processing workers compared to a non-exposed control group. However, there is little difference between exposed workers and controls in lung function parameters. The control group has an increased prevalence of self-reported asthma and allergy compared to the crab processing workers.

Furthermore, this work demonstrates elevated IgE in 8.9 % of king crab workers, while 17.5 % has positive skin prick test results. Among the edible crab workers, 12.2 % has elevated IgE to crab and 18.1 % has positive skin prick test results. Immunoblots also show that workers are sensitised to several allergens, including tropomyosin, arginine kinase, enolase and hemocyanin. Workers are sensitised and report respiratory which indicates they are at risk of developing occupational asthma and allergy.

The low prevalence of asthma and allergy despite the presence of respiratory symptoms and sensitisation suggests a healthy worker effect where unhealthy individuals are excluded from the workforce. It causes an underestimation of the health effects of working in the plant.

Preventive measures to limit the exposure through changes of the processing line or the use of personal protective equipment, and follow-up of workers' health should be implemented to prevent the development of occupational health problems among workers in the crab processing industry.

## Sammendrag

Yrkesrelatert astma og allergi er kjente helseutfordringer i sjømatindustrien. Det er flere faktorer som bidrar til utviklingen av luftveissykdommer, deriblant bioaerosoler som inneholder biologisk aktive agens. Disse blir inhalert av arbeidere under prosessereringen av sjømat. Gjennom dette arbeidet ønsket vi å undersøke norsk krabbeprosesseringsindustri og identifisere årsaksfaktorer til økt risiko for helseproblemer blant prosesseringsarbeidere.

Målet for denne avhandlingen var å undersøke bioaerosoler fra pustesonen til arbeidere som prosesserer krabbe. Vi ønsket å undersøke arbeiderne for tilstedeværelsen av luftveissymptomer, astma og sensibilisering for skalldyrallergener.

Avhandlingen beskriver eksponeringsnivåene av totalprotein-fraksjon, tropomyosin, trypsin og endotoksin i pustesonen til arbeidere i kongekrabbe- og taskekrabbefabrikker. I tillegg til dette beskrives eksponeringsnivå for NAGase hos taskekrabbearbeidere. Dette arbeidet viser at arbeidere som prosesserer kongekrabbe og taskekrabbe er eksponert for bioaerosoler som inneholder de overnevnte komponentene. Når man sammenligner prosessering av kongekrabbe og taskekrabbe har kongekrabbe prosessering høyest nivå av endotoksin, mens taskekrabbeprosessering har høyest nivå av tropomyosin og totalprotein. Arbeidsoppgaver og fabrikk var viktige faktorer for eksponeringsnivå. Eksponering for tropomyosin var høyest ved prosessering av kokt taskekrabbe, og lavest ved prosessering av kokt kongekrabbe. Trypsinaktiviteten var høyest ved prosessering av rå krabbe blant både kongekrabbe og taskekrabbearbeiderne. Forskjeller i eksponeringsnivå mellom kongekrabbefabrikkene tyder på en «fabrikk-effekt» hvor lokale forskjeller mellom fabrikkene i produksjon, ventilasjon og anleggsoppsett er viktig for eksponeringsnivå av bioaerosoler.

Dette arbeidet viser også at det er en økt tilstedeværelse av luftveissymptomer blant krabbeprosesseringsarbeidere sammenlignet med en kontrollgruppe ueksponerte arbeidere. Det var liten forskjell i lungefunksjonsparametere mellom krabbearbeidere og kontrollgruppa. Kontrollgruppa hadde mer selvrapportert astma og allergi sammenlignet med krabbearbeiderne.

Videre viser dette arbeidet at blant kongekrabbearbeiderne har 8.9 % forhøyet spesifikk IgE for krabbe og 17.5 % har positive prikktestresultat. Blant taskekrabbearbeiderne har 12.2 % forhøyet spesifikk IgE for krabbe og 18.2 % har positive prikktestresultat. Immunoblotting viser også at krabbearbeidere er sensibilisert for flere allergener, inkludert tropomyosin, arginin kinase, enolase og hemocyanin. Arbeidere som prosesserer kongekrabbe og taskekrabbe har en økt risiko for å utvikle yrkesastma og allergi.

Den lave prevalensen av astma og allergi til tross for luftveissymptomer og sensibilisering tyder på at det kan være en «healthy worker effekt» blant krabbearbeidere hvor de arbeiderne som ikke er friske blir ekskludert fra arbeidet. Dette fører til en undervurdering av helseutfordringene av å arbeide i krabbefabrikken.

For å redusere eksponeringen kan man gjøre forebyggende tiltak som å endre prosesslinja og ta i bruk personlig verneutstyr. Arbeidernes helse bør følges opp for å forhindre utviklingen av yrkesrelaterte helseproblemer.

## List of papers

The following papers are part of this thesis and will be referred to in the text by their Roman numerals:

- I. Kamath SD, Thomassen MR, Saptarshi SR, Nguyen HM, Aasmoe L, Bang BE, Lopata AL. Molecular and immunological approaches in quantifying the air-borne food allergen tropomyosin in crab processing facilities.  
Int J Hyg Environ Health. 2014 Sep;217(7):740-50. doi: 10.1016/j.ijheh.2014.03.006.
- II. Thomassen MR, Kamath SD, Lopata AL, Madsen AM, Eduard W, Bang BE, Aasmoe L. Occupational Exposure to Bioaerosols in Norwegian Crab Processing Plants.  
Ann Occup Hyg. 2016 Aug;60(7):781-94. doi: 10.1093/annhyg/mew030.
- III. Thomassen MR, Aasmoe L, Bang BE, Braaten T. Lung function and prevalence of respiratory symptoms in Norwegian crab processing workers.  
Int J Circumpolar Health. 2017;76(1):1313513. doi: 10.1080/22423982.2017.1313513.
- IV. Thomassen MR, Kamath SD, Bang BE, Lopata AL, Aasmoe L. Allergic sensitisation among Norwegian king crab (*Paralithodes camtschaticus*) and edible crab (*Cancer pagurus*) processing workers and identification of major allergenic proteins.  
*Manuscript.*

## Abbreviations

- AK – arginine kinase  
ATS – American Thoracic Society  
BCA - bicinchoninic acid assay  
BSA – bovine serum albumin  
CI – confidence interval  
COPD – chronic obstructive pulmonary disease  
ECL - enhanced chemiluminescence  
ELISA – Enzyme-linked immunosorbent assay  
EU – endotoxin units  
FAO - Food and Agricultural Organization of the United Nations  
FEV<sub>1</sub> – Forced expiratory volume in one second  
FVC – Forced vital capacity  
IgE – Immunoglobulin E  
IgG – immunoglobulin G  
IL – Interleukin  
LAL – Limulus Amoebocyte Lysate  
LOD – Limit of detection  
LPS – Lipopolysaccharides  
NIOH – National Institute of occupational health (STAMI)  
NAG - *N*-acetyl glucosamine  
NAGase - *N*-Acetylglucosaminidase  
OA – Occupational asthma  
OAI – Occupational allergy  
ODTS – organic dust toxic syndrome  
OR – odds ratio  
PBS – phosphate-buffered saline  
PBZ – personal breathing zone  
PPE – personal protective equipment  
PTFE - Polytetrafluoroethylene  
PVDF - polyvinylidene difluoride  
RPE – respiratory protective equipment  
SD – standard deviation  
SDS-PAGE - sodium dodecyl sulfate Polyacrylamide gel electrophoresis  
SPT – skin prick test  
TLR – toll-like receptors  
TM - tropomyosin  
TP – total protein  
TWA – Time-weighted average

## Definitions of concepts used in the thesis

**Bioaerosols** are particulate matter or liquid droplets suspended in air. They range from about 0.3-100µm in size. Bioaerosols contain agents of biological origin such as endotoxins, microorganisms, and proteins like high molecular weight allergens and enzymes depending on the type of seafood being processed (1). The movement of the bioaerosols in the air depends on their shape, size and density, as well as factors in the plant such as air currents and ventilation, humidity and temperature (2-4). Large bioaerosols settle fast due to gravitational forces, while particles in respirable range (aerodynamic diameter <10µm) are of particular concern because they stay airborne longer and may be inhaled and enter the respiratory system. Several specific steps of crab processing such as butchering, de-gilling, cracking, boiling, and washing/scrubbing have been shown to generate bioaerosols (5-8).

**“Crab asthma”** is the occupational asthma specifically to crab experienced by some crab processing workers. The term is mainly used with regard to snow crab processing workers in Canada (9, 10). Crab asthma is caused by components from the crab that enter the respiratory system of processing workers who then become sensitised. Common symptoms are chest tightness, cough, wheeze and shortness of breath. The symptoms may occur when working at the crab plant or in some cases after the shift has ended. When these symptoms are caused by an allergy to snow crab, the worker has occupational asthma to crab or “crab asthma”.

**Healthy worker effect (HWE)** is a selection process where unhealthy individuals are no longer part of the workforce (11-15). This selection of healthy workers leads to a difference in health status between workers and the general population and is therefore a source of selection bias in cross-sectional studies. As a consequence, HWE may obscure evidence of harm from hazards or cause an underestimation of the association between an occupational exposure and the disease. This has been found to be particularly important in studies of work-related asthma.

**Plant effect** is the effect of variations between different processing plants in parameters such as building layout, processing technology and worker behaviour (16-18). This will have an effect on the production, dispersion and removal of unwanted occupational exposures such as bioaerosols (19-21). It is the sum effect of layout of the processing line, processing equipment, handling procedures, amount of product being processed, variations in work procedures, ventilation and other building structures as well as other unmeasured covariates.



## 1 Background for the study

According to the Food and Agricultural Organization of the United Nations (FAO), over 58 million people world wide are involved in the primary sector of capture fisheries and aquaculture (22). Due to an increase in world population in general as well as an increased awareness of the health benefits of seafood, worldwide consumption of seafood products has increased. As a result, the international consumption and trade in shellfish has grown (23). Since the first published study in 1937 attributing occupational asthma to work in the seafood industry (24), several studies have found an increased prevalence and incidence of airway symptoms, asthma and allergy among seafood processing workers (5, 6, 9, 25-33). Reactions to occupational exposures can manifest in different ways, such as rhinitis, conjunctivitis, urticaria, protein contact dermatitis, asthma and systemic anaphylactic reactions (5). Sensitisation is documented in workers involved in processing fish, mussels, prawns and crabs, and workers in the shellfish industry have a higher prevalence than those in the bony fish industry (5, 6, 9, 34, 35). The prevalence of occupational asthma among workers exposed to shellfish is reported to be between 4 % and 36 % in different studies (5, 6, 9, 25, 26).

An important risk factor for the development of occupational health problems is the exposure to bioaerosols generated during seafood processing (1). Several specific steps of crab processing such as butchering, de-gilling, cracking, boiling, and washing/scrubbing have been shown to generate bioaerosols (5-7).

The observed respiratory health problems among production workers in the seafood industry may be caused by an allergic reaction, or by a non-allergic inflammatory process caused by inhalation of biological components such as endotoxins, moulds or proteases (36-38). Other workplace factors of non-biological origin such as saline, chemicals from cleaning, exhaust from vehicles and cold air may also trigger non-allergic respiratory symptoms (39, 40).

## 1.2 King crab (*Paralithodes camtschaticus*)

### 1.2.1 Background

In the 1960s the red king crab (also called Kamchatka crab) was introduced from the North Pacific to the Kola fjord in North-West Russia by Russian scientists as an attempt to establish a new source of food and commercial fishery (41). The crab thrived and migrated west (42), as was observed in the Norwegian magazine Fiskeribladet Fiskaren on January 20<sup>th</sup> 1977 where they published observations of the crab in Norwegian fjords next to the Russian border. Since the introduction of the king crab, it has become abundant along the Norwegian coast of Finnmark County which is the northernmost county of Norway with a border to Russia in the east (Figure 1).

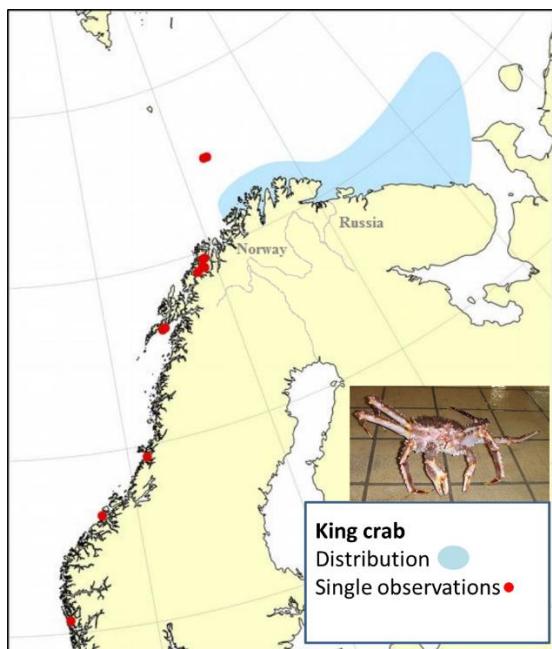


Figure 1 Geographical prevalence of king crab in June 2013. Photo adjusted from the Institute of Marine Research (<http://www.imr.no/temasider/skalldyr/kongekrabbe/nb-no>)

Commercial harvesting of king crab in Norway started in 2002, and in 2004 Norwegian authorities implemented an open-access fishery west of 26°E to minimize the expansion of the crab further west (43, 44). In 2016 the Directorate of Fisheries in Norway set the total quota for king crab at 2050 tons. King crab fishing season lasts from early autumn (around August) to mid winter (around January). The crabs are captured in pots and transported live to the shore. They are then bought by land based processing plants and either transported live or processed before they are sent to their final destination (45). The small quotas in Norway with a yearly catch of around 2000 metric tons and the small area of operation make these fisheries different

from king crab fisheries in other parts of the world. Still, the processing plants are of vital importance for several local communities in Finnmark (46).

### 1.2.2 Plant/processing

The processing plants are situated along the east coast of Finnmark County. Most plants are primarily fish processing plants with a temporary crab processing line placed in the factory during king crab fishing season. The different plants have different equipment for processing

the crab. Some have automated processing lines for some of the procedures while others rely mainly on manual labour.



Figure 2 Cracking



Figure 3 De-gilling



Figure 4 Cooking

The crabs are transported in large vats from the boats into the processing plant by trucks. The edible meat in the king crab is located in the legs. The first step in processing is “cracking” (Figure 2). The workers remove the clusters containing the crab meat (the two shoulder sections, each with three legs and a claw attached) from the carapace. The carapace is discarded while the clusters are further processed. Next the clusters are de-gilled. The gills that are attached to the shoulder sections are cleaned off (Figure 3) by rotating metal and plastic brushes, usually in combination with fresh water. After cleaning, the clusters are cooked (Figure 4) by lowering them into large cooking vats containing boiling fresh water. After cooking they are cooled by lowering into cold fresh water. Finally, they are glazed with water and frozen for storage and further transportation. Continually during processing, workers are also involved with cleaning floors, vats and processing equipment using water hoses, wipers and brooms.

### 1.2.3 Protective measures

The levels of measures taken to protect the workers varies between different plants.

Technologies used in the current plants ranged from mainly manual work with simple tools such as hand held brushes, to modern, highly automated processing lines. This affects both bioaerosol production and content (47-49). Little or no shielding of work tasks such as cracking and de-gilling leave the workers fully exposed to the particles produced during the processing. As most processing lines were temporary instalments, they rarely had optimal ventilation to remove bioaerosols. Some plants had placed the cooking vats in separate rooms or directly under point ventilation to reduce workers' exposure to steam from cooking. To protect their hands from the spikes on the crab shell, workers wore thick gloves. They also wore oilskins and boots to protect their clothing in the wet work environment.

## 1.3 Edible crab (*Cancer pagurus*)

### 1.3.1 Background

Edible crab (also known as Brown crab) is native to the Norwegian coast line and has been included in Norwegian fishery statistics since 1914. There are no quota regulations and in 2015, 4717 tons were collected. The distribution of the crab along the Norwegian coast stretches from the south up to Troms county (Figure 5) and the main fishing season is in late summer/autumn (August to November). The edible crabs are captured in pots and transported live to the shore. They are then bought by land based processing plants, processed and frozen before transportation to their final destination.

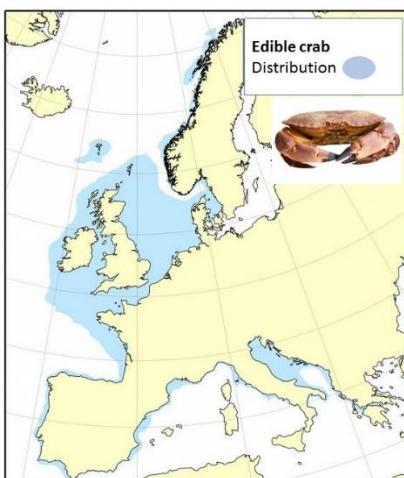


Figure 5 Geographical prevalence of edible crab in June 2013. Photo adjusted from the Institute of Marine Research (<http://www.imr.no/temasider/skalldyr/taskekrabbe/nb-no>)

### 1.3.2 Plant/processing

The plant processing edible crab is located along the coast of Mid-Norway. The crabs are transported live from the fishing vessels into the processing plant where they are anesthetized by icy water.



Figure 6 Processing raw crab



Figure 7 Processing cooked crab



Figure 8 Cleaning

From the cold water with ice, the crab is transported to the slaughtering station (Figure 6) where the whole crab or different elements of the crab (carapace, legs, claws) are cleaned, sorted and transferred to cooking vats or steamers. The work stations are designed so that several workers are stationed in close proximity to each other at each station. Most of the equipment used was not designed to shield the workers from the spray of the rotating brushes and water. After the cooked crab is cooled down, the different parts of the crab is further processed (Figure 7). Work tasks performed on cooked crab includes cleaning the carapace and large claws, emptying the legs and small claws of meat by cleaning, crushing and tumbling the pieces to utilize as much as possible of the whole crab in different final products. The extracted meat is collected and packed manually into the crab carapace, or it is sent for further processing. After final packaging, the crabs are frozen in large halls until transportation to buyers. During processing, some workers use water hoses (Figure 8), shovels, wipers and other equipment to clean transport vats as well as to remove dirt from the different processing equipment and the work surfaces and floor of the plant.

### 1.3.3 Protective measures

The edible crab plant included in our study was a large and well equipped plant with a high level of automation of the processing line. Conveyer belts transported the crabs between the different work stations including through the cooking/steaming process. Work tasks were poorly shielded. However, to reduce the accumulation of bioaerosols, the ventilation system inlets were placed close to the workers and directly above the cooking areas. Also, the steaming of most of the crab was performed on conveyer belts so the crab would be cooked with minimal need for workers to be stationed in the areas where the cooking fumes were let out. The workers wore gloves and plastic aprons to protect their clothing. Respiratory protective equipment (RPE) was available for those who wished to use them. Around one third of the workers did use RPE during some of the work tasks.

## 1.4 Health surveillance and exposure regulations in the seafood industry

The Norwegian Labour Inspection Authority monitor the compliance to regulations on organisation and management. Officially approved occupational health services monitor the health of workers in the seafood industry (Regulation on organization, management and participation §13-1). Their main tasks include helping the plant with risk assessment and implementing preventive measures to reduce the risk of ill health and accidents. Where risk assessments have concluded that monitoring workers' health is necessary, the occupational health service perform regular medical health controls targeting possible health risks for exposed workers. Spirometry measurements to facilitate early detection of respiratory diseases such as asthma are included in targeted medical examinations the occupational health service may perform. They may also suggest reassignment if workers are in danger of developing health problems, and follow up of plants, workers and management to ensure the best possible work environment. Occupational exposure limits exist for several substances in the work environment. However, there are presently no such limits for bioaerosol exposures relevant for the crab processing industry such as serine protease enzymes, endotoxins, total proteins and allergens.

## 1.5 Occupational exposures in the crab processing industry

The exposure to workers in the seafood industry varies greatly depending on the different types of seafood and the varying processing methods. Many work tasks in the seafood processing industry involve extensive use of water, and production areas are wet

environments with high relative humidity. The wet aerosols generated during manual production, machines or washing procedures are dispersed into the production area and may be inhaled by the workers (5, 6, 18, 34, 38).

In our study, we have focused on some of the components likely found in bioaerosols that are relevant exposure agents for development of occupational asthma and allergy from crab processing.

### 1.5.1 Total proteins

Proteins are polymers built of amino acids (50). They are the most versatile macromolecules in all living systems and are essential components in practically all biological processes. Proteins have several functions such as structural material, enzymes, transporters and antibodies. 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 (5-7, 34, 51-53). 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 necessarily a good indicator for specific components such as allergens (8, 18, 54), nor does it measure bioaerosols that are not protein based such as endotoxins. It is therefore necessary when looking at specific components in the bioaerosols to perform analyses meant for specific agents.

### 1.5.2 Allergens

Proteins that are harmless to most people may in some trigger a response from the immune system and cause an adverse reaction known as an allergic reaction. The protein that elicits this reaction is known as an allergen. After at least one encounter with the substance, the allergic person becomes sensitised – the antigens stimulate the cells of the immune system who recognises them as foreign. The antigens cause an allergic (hypersensitivity) reaction by a Type I (immediate hypersensitivity) immunoglobulin E (IgE) mediated reaction where the antigen stimulates B-cells and T-cells to proliferate and produce specific IgE antibodies to that antigen. The IgE binds to surface receptors of mast cells found in most tissues and basophils in the vascular system. Subsequent exposure to the antigen leads to lysis of the mast cells, and release preformed mediators such as histamine. As a result, several disease symptoms may appear such as rhino conjunctivitis, dermatitis, asthma and anaphylaxis (55,

56). Several studies in the crab processing industry have found that workers are exposed to allergens and that this exposure is associated with the development of occupational allergy and occupational asthma (crab asthma) attributed to working in the crab processing industry (21, 25, 28-30, 57). Some allergenic proteins are denominators of the allergic reaction in the majority of workers that show allergy to crab. If more than 50 % of the allergic subjects react to the allergen, it is termed a major allergen (58). Tropomyosin and arginine kinase are both major allergens identified in air samples from crab processing plants and found to be sensitising agents among crab processing workers (17, 59-62). In addition, several other proteins such as sarcoplasmic  $\text{Ca}^{+}$ -binding protein, myosin light chain, troponin C, triosephosphate isomerase and actin, have been identified as shellfish allergens (63, 64). However, since many studies do not identify the exact allergens causing the sensitisation, it is likely that there are several more allergens responsible for sensitisation. The processing procedures affect the reactions sensitised workers have to the allergens (65, 66). Different workers may react to different allergens (28), and IgE-based diagnosis for crab used in Norway is based on whole extracts of cooked edible crab meat (ImmunoCAP f23, Thermo Scientific) which may not include all allergens present in the whole crab.

### 1.5.3 Proteases

Proteases are important digestive enzymes. However, they are versatile and also display other functions such as multifunctional hormone-like signalling molecules. Proteases play a role in a number of physiological and pathophysiological events in the human body as 2-4 % of human genes encode proteases (67, 68). Proteases can be divided into five classes based on mechanisms of catalysis; aspartate proteases, metalloproteases, cysteine proteases, threonine proteases and serine proteases. One third of the proteases expressed are serine proteases, named after the serine residue at the active site of the enzyme. They are present throughout all cellular kingdoms in nature, including fish and crustaceans (36, 69). Some proteases regulate cell function by cleaving and activating protease activated receptors (PARs). This regulates pain and inflammation and affects several tissues (67), including causing contraction or relaxation of smooth muscle cells, and lung remodelling. PARs regulate the inflammatory response in the airways through recruitment of inflammatory cells. Inhaling bioaerosols containing serine proteases could therefore lead to an inflammation of cells in the respiratory tract through a non-allergic mechanism of airway symptoms (36, 37, 70-72). Trypsin is a serine protease shown to cause an effect in lungs by enzymatic proteolytic cleavage of PAR-2 that elicits a cellular signal transduction and cause inflammation (37, 73-75).

#### 1.5.4 NAGases

Chitin is a polymer of  $\beta$ -(1-4)-linked N-acetylglucosamine (NAG) that is the most abundant polysaccharide on earth after cellulose, and it is also a major component of most fungal cell walls, insect and crustacean exoskeletons (76, 77). Chitin is digested to NAG by two enzymes; chitinase and NAGase. NAGase is a widely distributed enzyme in nature and has important roles in e.g. molting cycle, digestion of chitinous foods and defence systems against parasites. It has been described in Green crabs (*Scylla serrata*) with a wide stability in both temperature and pH, demonstrating adaptability to changing environment (78). Since NAGase production is induced in the presence of chitin (79) it is expected to be found in bioaerosols in crab processing plants.

#### 1.5.5 Endotoxins

Endotoxins are part of the outer membrane of Gram-negative bacteria cells (80, 81). When all other chemical substances are removed and it is in a chemically pure form, it is known as lipopolysaccharides (LPS). LPS consists of a polysaccharide attached to a lipid (lipid A). The polysaccharide facilitates the solubility of the molecule in water, and is comprised of two parts; an oligosaccharide where composition varies between bacterial species and an invariable core section located between the oligosaccharide and the lipid A (82). The first definition of endotoxins as a heat-stable toxic substance was published in 1892 (83). In humans, endotoxins are recognised by pattern recognition receptor on the membrane called Toll-like receptors (TLR). TLR4 in a complex with CD14 and LPS-binding protein recognize and respond to endotoxin, causing the release of inflammatory mediators (82, 84). Crab processing plants are wet work areas that use water in processing procedures. This causes a continued high humidity which are optimal conditions for bacterial growth. Fungi grow on the non-dried materials and are released from damp materials (85). These bioaerosols will contain endotoxins that may be inhaled by the workers.

#### 1.5.6 Bioaerosols in crustacean processing

Airborne particles containing allergens have been found in several studies in the seafood industry in general (6, 18, 40, 86, 87). Table 1 show a summary of exposure assessments from crustacean processing. Work processes found to produce bioaerosols in crab processing are; butchering/grinding, cracking, de-gilling, cleaning and cooking/steaming and cleaning of the processing line or storage tanks with water hoses (5, 7, 17, 88).

Comparisons of studies assessing work exposures are difficult because of several factors, including different types of seafood being processed, the amount and the way they are being processed, and the number of workers involved. Cooking is not performed in all plants. Some freeze the raw crab, and some may process the whole animal without dividing it in pieces.

In addition to the seafood, the workers may also be exposed to other factors that may affect their health. The “plant effect” (see Definition of concepts, page V) may play a significant role as the size of the plant, the ventilation and equipment play a major role in both production, distribution and removal of bioaerosols. Natural spores, pollen and other components from the outdoor environment will also affect indoor air quality in any building. This will vary depending on time of day, time of year and building parameters such as open doors, windows and ventilation (89, 90).

Table 1 Quantitative bioaerosol exposure characteristics in crustacean processing industries

Industry	Protein levels µg/m <sup>3</sup>	Allergen levels ng/m <sup>3</sup>	Allergen presence IgE	Particulate levels mg/m <sup>3</sup>	Endotoxin levels EU/m <sup>3</sup>	NAGase pmol 4-MU/m <sup>3</sup>	Ref
Prawn				0.10-3.30			(49)
Shrimp		1500-6260			0.2-100.0		(38)
Rock lobster	LOD-1.97			LOD-0.66			(18)
Scampi		47-1042					(91)
Snow crab	LOD-6400 (area) 34-1500 (PBZ)		LOD-844 RAU/m <sup>3</sup>		1.1-949		(3)
Snow crab		53-547 (area) 179-5061 (PBZ)					(21)
Snow crab	0.07-0.88 µg/50mL blood sera		1.657 µg/50mL blood sera				(92)
Snow crab	1.10-5.16	3-115					(93)
Snow crab		Mean values AK 1.68-19.68 TM 2.26-20.34					(17)
Snow crab		3-602 (area) 19-3188 (PBZ)					(16)
Dungeness, snow and tanner crab		79-2504					(20)
King crab				0.14-0.176 (area)			(94)
King crab				0.03-0.160 (PBZ)			(95)
King crab*	0.3-48.0 (PBZ)	0.1-76.0 ng/m <sup>3</sup> (PBZ)			LOD-24000 (PBZ)		(8)
Edible crab*	2.4-97.5 (PBZ)	0.4-95.9 ng/m <sup>3</sup> (PBZ)			7-340 (PBZ)	69-3234 (PBZ)	(8)

LOD; limit of detection, PBZ; personal breathing zone, area; stationary measurements, AK; arginine kinase, TM; tropomyosin, RAU; relative antigen units,

\*; results from work included in this thesis. Modified and adapted from Jeebhay, M (6).

### 1.5.7 Other airborne exposures

Exposure factors in the workplace other than those from bioaerosols from processing such as preservatives (formaldehyde in fishmeal production, sodium disulphite) and spices (paprika and garlic) or other biological contaminants from organic dust may become aerosolised and inhaled (3, 96, 97). In addition, the exhaust produced by indoor use of vehicles running on propane and diesel fuel have been suggested as contributors to airway symptoms (38). Most vehicles used in crab processing plants are electric, reducing the amount of exhaust exposure, however some also use fossil fuel. In addition to particles from the product being processed, mold or other microorganisms growing in the moist environment may also become airborne and be part of the bioaerosol composition. Hygiene is an important focus area in food processing. This includes the use of disinfectants for cleaning the production areas. The use of disinfective foam that is used to cover surfaces often contain chlorine, ammonium and peroxygen compounds (98, 99). This is washed away with high pressure water. Cleaning processes are often performed by the workers, or it may be done by other cleaning personnel at night. Remnants of the cleaning and disinfecting products may be left in the workplace surfaces and air, and be part of the processing workers' exposure.

### 1.5.8 Physical environment

Ambient temperatures in the plants are often below 10°C. This may be caused by several factors, including the transport of products in and out of the plants. For the trucks to enter, large gates need to be opened and cold air can rush in. Crab fishing season is during autumn/winter where outdoor temperatures usually are below 10°C from August and between 0°C to -20°C from October/November to March on the coast of Finnmark. Additionally, large quantities of cold water is used in most work tasks. This water is spilled on the floor and working surfaces, cooling both the floor where workers stand and the work surfaces. Temperature requirements on the product being processed also lowers the ambient temperature in the plants. Often large freezers store the finished product. Trucks are used to transport the crabs into the freezer, letting out cold air through large gates that need to be open for the truck to pass. Cold work environment may have a negative effect on workers' health through triggering symptoms from muscles, skin and airways (39, 100).

Work tasks and intensity varies in the plants. Increase in physical activity increases the respiratory rate and thus the intake of cold air, bioaerosols and other airborne contaminants. Some tasks are light and includes sitting with minimal hand/arm movement (such as truck driving) while others work tasks are very strenuous with a lot of movement (such as

cracking). Some work tasks rely entirely on manual handling of the products, while other tasks involve machines (53). While the use of machines reduce the direct contact with the food, it has a potential for greater bioaerosol production. Some processes may also produce dry particles that are released into the air, such as dry salt particles or other chemical additives, exhaust particles from forklifts or other fuel based machinery (38, 49, 97). The plant effect (see Definitions of concepts, page V) has also been found to play an important role in exposure among workers processing shellfish (8, 16, 18).

## 1.6 Occupational health in the crab processing industry

Bioaerosols generated during seafood processing is associated with respiratory health problems in workers inhaling these particles. The symptoms workers experience have usually been assessed by a questionnaire. Possible mechanisms for the development of symptoms were explored by immunological testing such as skin prick tests or specific IgE in blood samples. Irrespective of the type of seafood being processed, the prevalence of symptoms found in various seafood industries are high. However, the prevalence of allergy has been found to be higher in workers processing crustaceans compared to bony fish (5, 7). The association between working with crab processing and the development of respiratory symptoms and crab asthma has been studied since the 1970s when the first publications from Canada and Alaska came out (32, 94). Research published in the 1980s in both USA (31) and Canada (29, 101) found symptoms from upper and lower airways in crab processing workers, ranging from mild to severe. A summary of several published studies on crab processing workers is listed in Table 2.

Table 2 Studies and case reports of occupational asthma and allergy due to crustaceans

Agent	Subjects	Symptoms	Asthma (A) Occupational asthma (OA)	Skin prick tests	Immunological tests	Ref
Prawn	50	Respiratory symptoms 18/50 reduced lung function 12/50 dermatitis		+ 26 %	+ tIgE 20/50 + sIgE prawn 8/50 + prawn agar gel 30/50	(49)
Shrimp	1	Urticaria Anafylaxis		+ shrimp and herring	+ sIgE herring, sardine, shrimp and swordfish	(102)
Gammarus shrimp	1	Dyspnea Respiratory symptoms	OA	+ dried Gammarus	+ RAST and immunoblot to Gammarus	(103)
Shrimp	60	Respiratory symptoms			+ tIgE 13.6 %, + sIgE to shrimp 20.3 %	(38)
Brine shrimp	24	Respiratory symptoms Skin symptoms 17 %		+ 17 %	IgE antibodies 21 %	(104)
Shrimp shell powder	1	Respiratory and flu-like symptoms (ODTS)	Negative		Normal IgE Elevated IgG	(105)
Shrimp and scallops	1	Urticaria	OA	+ shrimp and scallops	+ immunoblots raw meat and cooked water from shrimp and scallops	(106)
Shrimp and clam	57	rhinoconjunctivitis shrimp 5 % clam 7 %	Total A 26 % OA Shrimp 4 % OA Clam 4 %	+ shrimp 16 % + clam 5 %	+ sIgE shrimp 14 % + sIgE clam 7 %	(27)
Lobster	1	Respiratory symptoms to sodium disulphide	OA			(97)
Norway lobster	52				Elevated IgE compared to controls	(107)
Snow crab	107	Rhinitis	A 2 % incidence/6 weeks OA 33/46 positive specific provocation		Normal IgG compared to controls + RAST 6 -8 %	(4)
Snow crab Atlantic shrimp	20	Asthma-like symptoms Rhinitis Conjunctivitis Skin rash	Probable OA 11 %	+ snow crab 40 % + shrimp 20 %	+ tIgE 10 % + sIgE snow crab 21 %	(108)
Snow crab	215	Rhino-conjunctivitis Skin Rash	OA 15.8 %	+ 18.3 % of tested (n=164)	+ sIgE crab 14.3 % of tested (n=196)	(30)

Agent	Subjects	Symptoms	Asthma (A) Occupational asthma (OA)	Skin prick tests	Immunological tests	Ref
Snow crab		Respiratory symptoms Wheeze 12.2 %	A 10.2 %			(109)
Snow crab	207				+ sIgE crab 39/207 IgE binding to multiple proteins 22/24	(28)
Snow crab	215		OA 18 % highly probable 22 % possible		Occupational allergy highly likely 18 % Occupational allergy possible 16 %	(9, 16)
Snow crab	119		OA 17.8 %	+ crab cooking water 54/110 + crab meat 56/110	+ RAST crab cooking water 52/110 + RAST crab meat 39/111	(101)
Snow crab	303	Rhino-conjunctivitis Skin rash				(29)
Queen crab	69	Rhinitis/hay fever 17/69 Hypocalcemia		+ mixed antigen 4/17 + crab 8/17		(32)
Dungeness, king, snow and tanner crab	82	Respiratory symptoms 33 % new onset				(20)
King crab	825	NA	A 1.5 % incidence			(3)
King crab	186	Respiratory symptoms Lung function	A 13 %		+ sera precipitin bands 9/15 + intradermal skin test 9/15	(94)
King crab*	139	Respiratory symptoms Lung function	A 9.9 %			(110)
Edible crab*	70	Respiratory symptoms Lung function	A 3.2 %			(110)

+: positive result, tIgE; total IgE, sIgE; specific IgE, \*; results from work included in this thesis. Adopted and modified from Jeebhay M (6)

### **1.6.1 Airways**

It is estimated that occupational factors account for almost 17 % of adult cases of asthma (111-113). It is the most frequent work-related respiratory disease in the seafood industry (5) with a prevalence between 4 % and 36 % among shellfish processing workers (5, 6, 25, 26). Studies in the seafood industry have also found workers with impaired lung function, respiratory symptoms and runny and itchy nose and eyes without specific sensitisation to allergens (4, 29, 30, 32). The symptoms may be caused by agents that act as irritants or bind to surface receptors linked to inflammatory airway responses (5, 38, 87, 114).

In addition to components in the bioaerosols, other factors such as cold air trigger nasal symptoms, cough, bronchial constrictions and asthmatic attacks (39, 115-117). Disinfectants have also been found to have an irritative effect on the airways as well as acute irritative symptoms in eyes, nose and throat (99, 118). Inhalation of LPS has been shown to produce symptoms such as chest tightness, cough, dyspnea, headache, joint and muscle pains and tiredness. In addition to this, it can produce airway inflammation, asthma symptoms, bronchial obstruction and diseases such as Organic Dust Toxic Syndrome (ODTS) and allergic alveolitis (1, 53, 80, 81, 85, 119).

The time from start of exposure to development of symptoms varies from weeks to years, but symptoms are typically worst during work and improve during weekends and holidays (120). Since rhinitis and conjunctivitis may be precursors for asthma (120, 121), these symptoms may be used as an early marker for risk of occupational asthma and may be a useful indicator to implement preventive measures on symptomatic workers. Removal from exposure usually results in improvement of symptoms, but with a plateau where symptomatic workers do not improve further (122-124). The duration of exposure after symptoms occur is important for the workers chance of improvement.

### **1.6.2 Allergy**

Several studies in the crab processing industry have found that workers are exposed to allergens and may develop occupational sensitisation or allergy (21, 25, 28-30, 57). The asthmatic reactions found in crab processing workers are predominantly IgE-mediated (5, 23, 101, 125, 126). Studies in the snow crab industry reported occupational allergy or sensitisation in 9-42 % of workers processing crab (9, 30). The allergic reaction may cause reactions ranging from rhinoconjunctivitis or small irritations on the skin, to more severe reactions such as asthma, alveolitis and anaphylaxis. The symptoms may come as an

immediate reaction minutes after exposure, or there may be a late phase reaction hours after the exposure (29, 63, 127). The late phase reaction may come after the workers have left the exposure areas and may lead the workers to misjudge the cause of their symptoms since the exposure is not present when the symptoms occur.

### 1.6.3 Contributing factors

Even though there currently is no known method of accurately predicting which worker will become sensitised or develop occupational health problems, there are factors that may increase the risk. Host associated risk factors such as gender and atopy and have sometimes been found to be significant risk factors for seafood processing workers for developing occupational asthma and allergy (25, 30, 125, 128, 129), but not in all studies (29, 86). Asthma and atopy are related conditions and involve both environmental and genetic factors and are therefore difficult, but important, to take into consideration (130). Several studies, including in the crustacean industry, also find smoking to be a significant factor for developing occupational asthma (29, 107, 122, 131).

Studies among seafood processing workers have indicated an exposure-response relationship between bioaerosol exposure and development of health problems (30, 40). A study on salmon workers found an association between total protein exposure and self-reported cough and chest tightness as well as cross-shift decline in FEV<sub>1</sub> on Mondays. The workers also showed a gradual adjustment to the exposure throughout the work week resulting in the most pronounced effect seen on Mondays (40). In prawn production, transferring from using compressed air jets to water jets resulted in a decrease of both airborne particles and workers' symptoms (49). A Canadian snow crab processing plant found an association between cumulative exposure (both duration of work at the plant and level of exposure) and occupational asthma and allergy (30). Exhaust particles from vehicles such as forklifts used inside the production areas of processing plants have also been found to have an effect on respiratory health (38, 132).

## 2. Rationale for the thesis

It is important to understand the hazards at the work site, the central exposure and its effects on workers' health. In the seafood industry, levels of technology varies greatly between countries as well as between processing plants within each country. The effect of new technology on bioaerosol production and dispersion should be examined. Few studies have been done on exposure during king crab and edible crab processing. Knowing the exposure is important when assessing workplace layout and development of health problems. The components in bioaerosols produced during different work tasks needs to be characterised. Conditions facilitating the release of allergens, enzymes and other components should be linked to work tasks. The effect these components have, whether alone or in combinations, should be found to assess their contribution on the development of occupational diseases such as occupational asthma and allergy.

In Norway, 69.8 % of the population are working (133). Of those not working, 359000 people have left for early retirement, or are deemed unfit to work because of their health. This group cost the Norwegian government 389 billion NOK in 2015 (134). In the Norwegian population, 15 % of the adult onset asthma is attributed to occupational exposure however there is likely a large degree of underreporting due to a lack of awareness and experience among doctors (135). Work is a key factor in a person's self worth, identity and participation in the community (136). It is therefore central to improve the health of our work force. By doing this, we ensure a healthy life while people are at work and in old age, we promote social inclusion and keep the knowledge and the competence in the work place. An early intervention is important to prevent absence that may lead to long-term sickness and possibly unemployment (136).

The development of occupational health problems may be avoided and a healthy working population sustained through identifying work place hazards in crab processing plants and finding ways to reduce the impact of the work environment on crab processing workers' health.

### 3 Aims

The general objective of the study was to gain knowledge of the bioaerosol exposure and health status of processing workers in Norwegian crab processing plants. With this knowledge it is possible to implement protective measures to prevent the development of occupational health problems.

- The specific aims were to:
  - find determinants of exposure and personal exposure levels to central components in bioaerosols produced during crab processing
  - contribute to the development of methods for quantifying allergens in the personal breathing zone of workers exposed to bioaerosols
  - examine the respiratory health status of crab processing workers in land based processing plants compared to a control group of workers not exposed to the seafood industry through self-reported respiratory symptoms and diagnoses, spirometric results and host-associated factors
  - examine the sensitisation status of crab processing workers through IgE testing and find possible determinants of allergic sensitisation through immunoblotting
  - make a knowledge base as a first step in the development of preventive strategies to reduce the occupational exposure to components causing occupational health problems

## 4 Study population and methods

### 4.1 Background

In 1999 – 2001, a study was conducted at the Department of Occupational and Environmental Medicine at the University Hospital of North Norway where an increased prevalence of respiratory symptoms were found among seafood processing workers compared to administrative workers in the same plants (38). This study included processing workers in the white fish, shrimp, herring and salmon industry. Further studies in the salmon industry were conducted in 2007 – 2010 and compared salmon processing workers to a control group of municipal workers (35, 40). In these studies, exposure measurements from the workers' breathing zone were also collected and analysed for bioaerosol components. The studies described an increased prevalence of respiratory symptoms, impaired respiratory status and increased sensitisation to seafood compared to the controls. They also reported exposure levels of proteins, parvalbumin and endotoxin in the workers' breathing zone. An exposure-response relationship was found between exposure to total protein fraction and respiratory symptoms and lung function test outcomes.

In 2002 the Norwegian government opened for commercial fishing for king crab and the result was that several fish processing plants along the coast of Finnmark county, in addition to fish processing, started to process king crab in crab fishing season during September – January. The prevalence of health problems such as occupational asthma and allergy is higher in crustacean processing industry compared to bony fish processing (6, 27, 28). On this background we chose to explore the occupational exposure and associated risks for occupationally related health problem in this new and growing industry. A well established industry of edible crab processing has existed since 1914. These are also crab processing workers and are likely exposed to many of the same components, but process a different crab, use a different processing line and may therefore have some different challenges. To compare this established edible crab industry to the new and growing king crab industry could demonstrate differences between the types of crab being processed or processing techniques that may explain possible differences in occupational health outcomes.

## 4.2 Study populations

Because of unpredictable processing schedules of catch and delivery of crab, and a large turnover of processing workers, a cohort study was not possible and the data collection was set up as a cross-sectional design.

**Ethical considerations;** The study was approved by the Regional committee for Medical Research Ethics in North Norway. Written information were given to all potential participants with information about the study and the data collection (Appendix A and B for controls and crab processing workers respectively). It also contained information on the anonymity of their answers and that no personal information would be forwarded to the employers or any other person other than the project leaders. Contact information to the project leader and the PhD student was included so that any workers who had questions or wished to retract their participation could do so. Written informed consent to participate was obtained from all participants in the study (Appendix A and B for controls and crab processing workers respectively).

### 4.2.1 King crab workers

Data was collected between September 2009 and November 2011. The king crab fishing season starts at the end of summer and continue to January when the crabs start molting.

Recruitment of king crab processing plants was based on a list of plants buying king crab registered at The Norwegian Fishermen's sales organization in 2009. The participation is illustrated in Figure 9 and Figure 10. Of the 20 plants identified, 14 of these plants still had production and wished to participate in the study. Based on size (at least 12 workers) and location (convenient to access the plant with the necessary equipment), four plants were included in the exposure measurements and health examinations. Three of the plants had crab processing during our visit, the fourth plant participated only in the health examinations. The remaining 10 plants were not included in the exposure measurements and health examinations, but participated in the questionnaire study.

A contact person was chosen at each plant. The contact person was responsible for distribution of information, consent papers and questionnaires to all employees in their plant.

Due to an unexpected abruption of the king crab season in 2010, the response rate from the plants who only participated in the questionnaire study was 23 %. At least one worker returned the questionnaire in 8 of the 10 plants who received the forms.

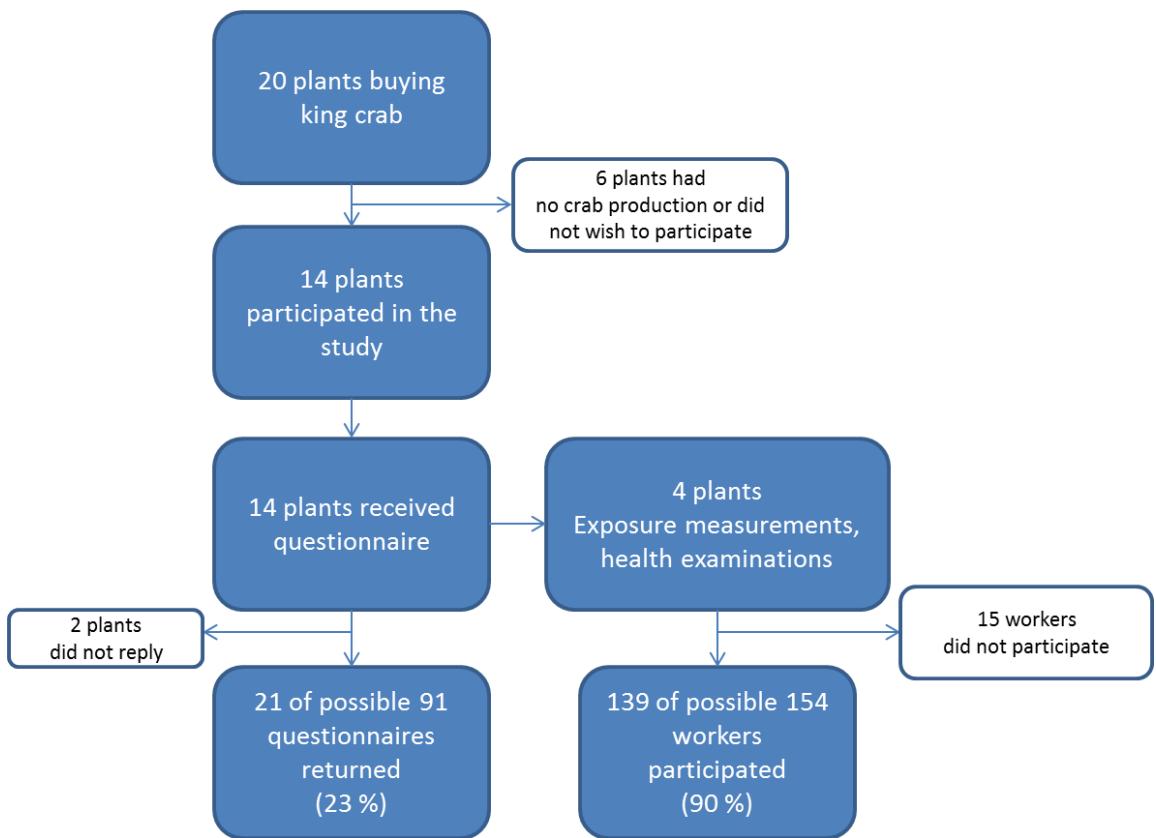


Figure 9 Participation of king crab plants and workers in the study

The king crab processing group included in the health examinations consisted of 154 workers from four plants where 139 workers (90 % of the eligible work force) participated in one or more of the examinations (Figure 10). There were no requirements to have answered the questionnaire to participate in the health examinations or vice versa so some workers participated only in the health examinations while others only answered the questionnaire.

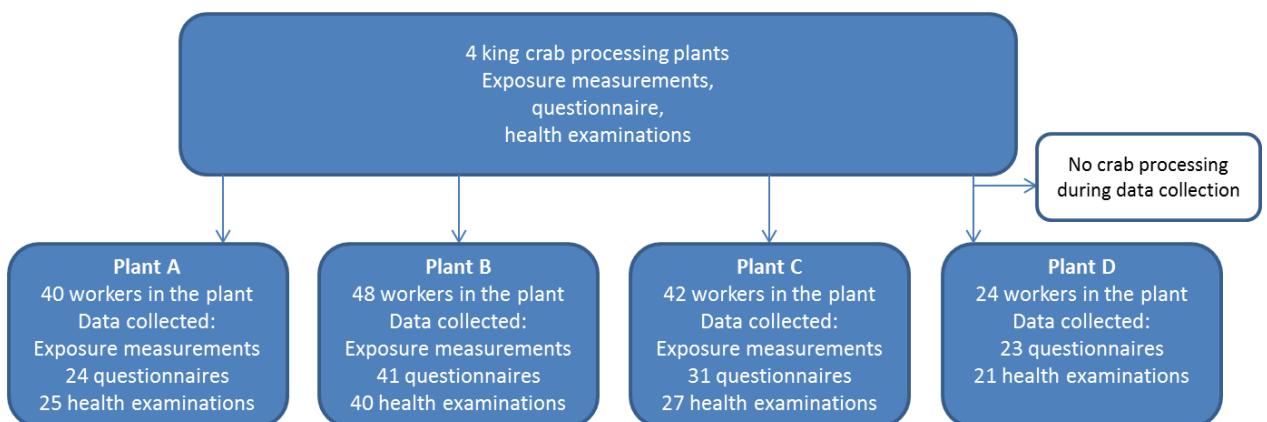


Figure 10 Participation of workers from the different king crab plants in the study

#### 4.2.2 Edible crab workers

Data was collected in September and October 2011. The main edible crab fishing season starts around August and continue to November.

The Department of Occupational and Environmental Medicine was contacted by the manager of the edible crab processing plant wishing to participate in the study. Since this plant was the only edible crab processing plant large enough to include the exposure measurements, they were the only edible crab plant included.

A contact person in the plant was chosen to distribute information, consent papers and questionnaires to all employees.

The edible crab processing group consisted of 89 workers where 83 (93 % of the eligible work force) participated in one or more of the examinations, see Figure 11. Thirteen of the workers did not work 50 % or more in crab processing areas and were therefore not included in lung function measurements.

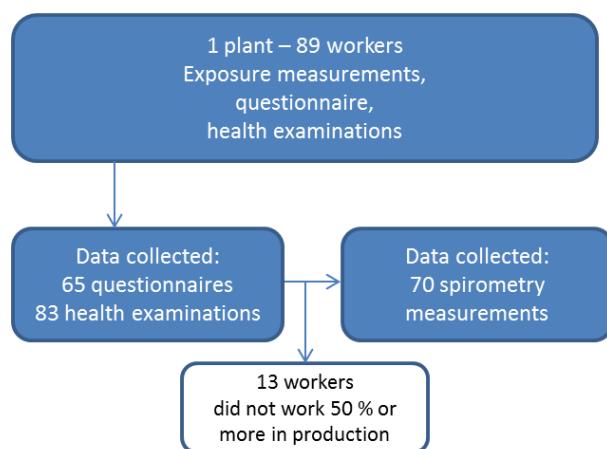


Figure 11 Participation of edible crab plants and workers in the study

#### 4.2.3 Non-exposed control population

Data was collected between November 2007 and April 2008. This control population has been used in a previous study in the salmon processing industry (35).

The control population consisted of people working in administrative organisations and schools in four coastal communities. To be included in the study, the workers had to be 18 years or older and be employed in at least 80 % position. Workers were excluded if they had previously worked in any kind of seafood industry.

As with the crab processing workers, a contact person was selected at each municipality to distribute the questionnaire and consent forms. An excess of questionnaires were sent to the contacts. There is no information on how many employees were asked to participate so the response rate in the control group is unknown. In total, 215 workers answered the questionnaire. Not all workers who answered the questionnaire wanted to or were available to participate in the health examinations, so of the 215 workers who answered the questionnaire, 151 (70.6 %) participated in the health examinations (Figure 12).

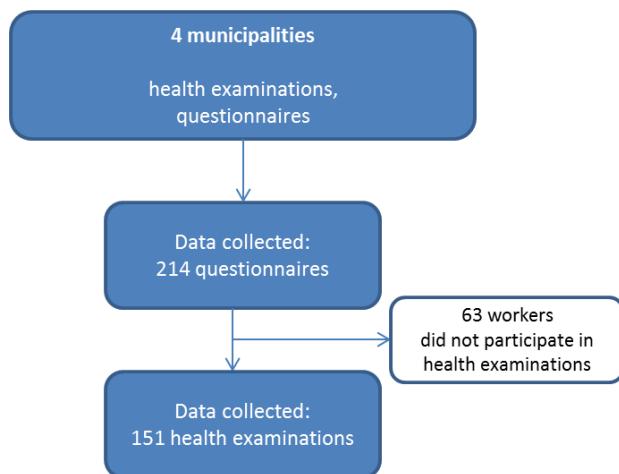


Figure 12 Participation of non-exposed control group workers in the study

## 4.3 Methods for data collection from workers

### 4.3.1 Questionnaire

The questionnaire was distributed in Norwegian and English (Appendix C and D for controls and crab processing workers respectively). One king crab processing plant who only participated in the questionnaire study had workers who did not speak Norwegian. These workers received the questionnaire in English. All other participants answered the questionnaire in Norwegian. In the plants participating in the health examinations, questionnaires were available in both Norwegian and English, and the workers could choose the language they preferred. Contact information was given along with the questionnaire in case the workers had any questions. In the plants who participated in both the questionnaire and health examinations, the workers could get assistance from a member of the research group when answering the questionnaire.

The questionnaire was based on previous studies in the seafood industry (35, 38, 137). Questions about the workers general respiratory symptoms (wheezing, shortness of breath, shortness of breath with wheezing, daily morning cough, daily morning cough with phlegm

and prolonged cough) were based on a modified version of a questionnaire developed by the British Medical Research Council (138). The questionnaire also contained questions on personal and family history of diseases. In addition to this, general demographic information such as age and gender was also collected. All questions on respiratory symptoms were limited to the last 12 months.

Questions derived from Scandinavian studies on organic dust-related respiratory effects (139, 140) focused on health problems the workers themselves related to their work. Due to many missing values in questions regarding symptoms attributed to work, these were not included in the statistical analyses. There was no clear reason why the workers chose not to answer this section, nor was this experienced in previous studies with similar questionnaires.

#### 4.3.2 Spirometry measurements

Spirometry measurements were performed using a SpidaUSB (CareFusion 234 GmbH, Hoechberg, Germany). Workers were instructed not to smoke for two hours before testing, but no restrictions were made on use of asthma medication. Notes were made on the use of medication in the Spida software. The forced expiratory volume in the first second of exhalation (FEV<sub>1</sub>) and forced vital capacity (FVC) were measured by instructing the person to expire forcefully after a full inspiratory maneuver. This was repeated until the test satisfied the American Thoracic Society 1995 criteria (141) but no more than 8 times. The highest values of FEV<sub>1</sub> (L/s), FVC (L) and FEV<sub>1</sub>/FVC (%) were retained for analyses. To calculate predicted lung function values, data were collected on gender, age, and height. Calculations of the predicted values were based on Langhammer et al (142) for a non-smoking Norwegian adult population. Reduced lung function was classified by FEV<sub>1</sub> and/or FVC of less than 80 % of predicted values. To limit the effect of age on airway obstruction, FEV<sub>1</sub>/FVC below the 5<sup>th</sup> percentile of the predicted values was characterized as airway obstruction (143, 144).

#### 4.3.3 Skin prick tests

Skin prick test (SPT) were performed on crab processing workers on the ventral aspect of the forearm, and reactions were read after 15 minutes. SPT reactions were read as positive if the extract caused a wheal of  $\geq 3$  mm in the presence of a positive control of 1 % histamine solution, and no response to the negative control of 0.9 % saline solution (Soluprick, ALK-Adellö AS, Denmark). In addition to the positive and negative controls, in-house crab extracts generated at the Department of Medical Biology at UiT the Arctic University of Norway were used; king crab extracts on king crab production workers and edible crab extracts on edible

crab production workers. Raw and cooked king crab and edible crab was purchased commercially from crab production plants. Four separate extracts were made; raw meat, cooked meat, intestines (raw crab) and shell (raw crab) (28). Each component was blended with PBS and centrifuged (10 000g for 1h). The supernatant was further centrifuged (80 000g for 1h), and the protein content in this supernatant was assayed by the Bradford method (145). These solutions were defined as the final crab extracts and were used for SPT as well as immunoblotting. The protein concentrations in the final king crab extracts were 2.7 mg/ml, 0.5 mg/ml, 5.9 mg/ml and 4.21 mg/ml in raw meat, cooked meat, intestine and shell extracts respectively. The protein concentrations in the final edible crab extracts were 1.8 mg/ml, 2.5 mg/ml, 2.4 mg/ml and 1.9 mg/ml in raw meat, cooked meat, intestine and shell extracts respectively. The extracts were aliquoted in 1 mL samples and stored at -80°C until used.

#### 4.3.4 Blood samples

Blood samples were collected in BD Vacutainer serum separation tubes (Thermo Fisher Scientific), centrifuged and the serum collected. Serum was stored refrigerated until arrival at the laboratory where they were stored at -80°C until analysed.

#### IgE analyses

The IgE levels of the control group, the king crab and edible crab workers were all analysed at the Department of Laboratory Medicine at the University Hospital of North Norway. The atopy status was established by quantifying specific IgE to 10 common inhalant allergens (birch, timothy, wormwood, alternaria, cladosporium, cat, horse, dog, house dust mite and rabbit). The detection of specific IgE  $\geq 0.35$  kU/L to at least one of the common inhalant allergens was used as a positive result for atopy.

In addition to this, crab workers' serum were analysed for specific IgE to crab using the ImmunoCAP system (boiled crab meat from *Cancer pagurus* code f23, Thermo Scientific). Crab processing workers with specific IgE  $\geq 0.35$  kU/L to crab were defined as having elevated IgE to crab. This could not be performed on the control group as the blood samples were no longer available.

#### Immunoblots

Blood samples from the 10 edible crab workers and the 10 king crab workers with highest specific IgE to crab in the ImmunoCAP analysis were used for immunoblotting. The four extracts of the relevant crab species were used (see section 4.3.3). These made it possible to

study the workers' serum IgE antibody binding patterns to allergenic proteins in the different crab extracts. Immunoblotting was performed at the laboratory of Andreas Lopata at James Cook University in Townsville, Australia. The crab extracts were resolved on a SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane where they were incubated with worker sera. The binding of the worker IgE was visualised and semi-quantified as low, medium or high binding and allergograms were generated to compare the workers' IgE antibody binding patterns.

To confirm IgE binding to two known major allergens in crustaceans, tropomyosin and arginine kinase (17, 61, 146, 147) as well as two novel allergens hemocyanin and enolase, the SDS-PAGE gel bands for these two allergens were excised and characterised (61, 148-150) at the molecular level using mass spectrometry at James Cook University, Australia. The allergen hemocyanin, previously studied as a shrimp allergen (151) and in crab roe (152) was identified in the intestine extracts. The novel shellfish allergen enolase (153) was also identified in the intestines as well as in the raw meat extracts.

## 4.4 Methods for exposure measurements

### 4.4.1 Personal air sampling

At each of the processing plants where exposure measurements were taken, 12 exposed workers from central areas in the processing line were chosen. Each worker wore a backpack

containing air sampling equipment (Figure 13) consisting of 3 sampling pumps sampling air through a filter cassette connected to the sampling pump through a sampling tube. To prevent the sampling tube being bent or flattened, they were reinforced with a hard outer tube. Personal exposure measurements were performed throughout the work shifts on the days

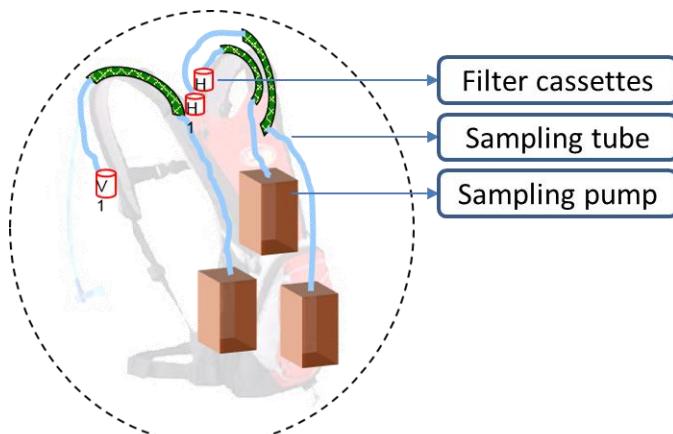


Figure 13 Backpack for air sampling equipment

of production using SKC Sidekick (SKC Ltd., Dorset, UK) sampling pumps. Air flow rates were set to 3.0 L/min for tropomyosin, total protein, trypsin and *N*-Acetylglucosaminidase (NAGase), and 2.0 L/min for endotoxin. 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 (when the equipment was collected from the workers). Each backpack contained three sampling pumps connected to filter cassettes. The air samples collected from workers' personal breathing zone (PBZ) were analysed for airborne total protein (TP), the major allergen tropomyosin (TM), trypsin-like enzyme activity and endotoxin. Additionally, samples dedicated to NAGase analyses were collected at the edible crab plant. Endotoxin samples were collected on glass fibre filters (Whatman GF/A, Kent, Maidstone) using PAS6 cassettes (Personal Air Sampler with 6 mm inlet) manufactured at the National Institute of Occupational Health in Oslo, Norway. The rest of the samples were collected using SureSeal Air Monitoring Cassettes (37 mm, 3-pc, styrene SKC Ltd. UK) on polytetrafluoroethylene (PTFE/Teflon) filters with polypropylene support (37 mm, 1.0 µm SKC Ltd. UK). NAGase samples were collected using polycarbonate filters using SureSeal Air Monitoring Cassettes. After use, the cassettes were cleaned externally with 70 % ethanol. Tropomyosin, total protein, trypsin and NAGase cassettes were stored at -20°C, and endotoxin cassettes at +4°C until extraction. The workers who had carried sampling equipment also registered work tasks during the shift.

#### 4.4.2 Total protein analyses

The protein filters were extracted in 1.0 mL phosphate-buffered saline (PBS) with 0.05 % Tween 20. Samples were transferred to mini eppendorf tubes and stored at -70°C. Manual QuantiPro bicinchoninic acid assay (BCA) Kit (Sigma-Aldrich, St. Louis, USA) was used to determine levels of total protein ( $\mu\text{g}/\text{m}^3$ ) in the samples by colorimetric reading of  $\text{Cu}^{1+}$ -BCA complex in a spectrophotometer at 560 nm (154). Analyses were performed at the Department of Medical Biology at UiT the Arctic University of Norway.

#### 4.4.3 Tropomyosin analyses

The filters were extracted in 1.0 mL PBS with 0.5 % Tween 20 and  $\text{NaN}_3$  for conservation, transferred to 1mL mini Eppendorf tubes with bovine serum albumin (BSA) and frozen at -20°C until analysed. The tropomyosin analyses employed an ELISA sandwich method described by Lopata et al (155). Purified recombinant tropomyosin was used as the allergen standard. A high binding Costar microtitre plate (Sigma Aldrich, USA) was coated with anti-tropomyosin anti-body in carbonate buffer (pH 9.6) and incubated over night. 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. 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. Analyses were performed by co-operators at the Centre for Biodiscovery and Molecular Development of Therapeutics at James Cook University, Australia.

#### 4.4.4 Endotoxin analyses

The filters were analysed by a quantitative kinetic chromogenic Limulus Amoebocyte Lysate (LAL) assay (156) and results are expressed in EU/m<sup>3</sup> (EU = endotoxin units, 10 EU≈1 ng endotoxin). The glass fibre filters were extracted in 5 mL LAL water with Tween 20 and stirred at room temperature for 1h. The samples were then centrifuged at 1000 G and distributed in several non-pyrogenic tubes and frozen until analysis. Air samples were placed on a non-pyrogenic micro plate and LAL lysate added. The clotting enzyme present in the LAL lysate splits p-nitroaniline which causes a yellow coloration that is read by photometric measurements at 405 nm. Analyses were performed at the Norwegian institute of occupational health in Oslo.

#### 4.4.5 NAGase analyses

Introduction of NAGase activity was done at the end of the sampling period and was therefore only systematically performed in 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) (157). 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 (158). Analyses were performed by co-operators at the National Research Centre for the Working Environment in Copenhagen, Denmark.

#### 4.4.6 Trypsin analyses

Trypsin-like activity in filter extracts were analysed by means of zymography. Five µL sample extracts were applied on zymographic gels (Novex® no.EC61752, ThermoFisher Scientific) containing gelatine as protease substrate. A standard curve (0.014 - 0.228 mU/mL) was prepared by dilution of a porcine trypsin stock solution with known enzyme activity. Trypsin standards and aliquots of filter sample extracts were mixed with loading buffer (Novex®, ThermoFisher Scientific) and the gel was subjected to electrophoresis at 20 mA/gel

for 2 hours. Thereafter, the gel was washed and incubated over night in developing buffer at 37 °C (Novex®, ThermoFisher Scientific) and stained in 0.2 % Coomassie Brilliant Blue R-250 Dye. The activity of gelatine degrading proteases were detected as clear zones against the undigested, stained background. The intensity of zymographic 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 trypsin inhibitor aprotinin. Together with the band size this strongly suggests that the protease activity in this region is due to trypsin (159).

## 4.5 Statistical analyses

Analyses were performed using GraphPad Prism version 6.02 (GraphPad, USA) (paper I), SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 22 and 23 (paper II and IV), and Stata/SE 13.0 (StataCorp LP, Texas, USA) (paper III). Two sided P values < 0.05 were considered statistically significant. The statistical procedures are described in the respective papers.

Regression and logistic regression analyses with adjustments for potential confounders were used to calculate  $\beta$  coefficients and odds ratio on outcomes of interest (paper III). There risk of missing confounders can not be completely excluded. However, an extensive knowledge of research in the seafood industry among the senior researchers as well as a thorough preparatory work minimises the chance of there being important factors not taken into consideration. In situations where few subjects had the outcome of interest, regression analysis may cause biased estimations so results need to be interpreted with caution. Most analyses were performed using crab species stratification, although in paper III some analyses were performed on all crab processing workers combined to gain statistical power. In paper III, as lung function parameters were missing at random, multiple imputation was performed to improve efficiency (160). The complete regression model was applied throughout the imputation process and no evidence was found of systematic differences between the imputed and non-imputed data. This is a method often used on data with randomly missing values to prevent whole subjects being excluded from the analyses because of a missing variable. By imputing the missing variables, the confidence intervals (CI) often decrease and power increase as the number of subject included in the analyses increase compared to the non-imputed dataset.

Results from the exposure measurements had a skewed distribution and so was log transformed before statistical analyses were performed to achieve normal distribution (paper II). Skewed distribution of exposure measurements are common and the geometric mean is considered to be a better representation of the data than arithmetic mean because of the smaller impact of extreme values. Mann Whitney U tests were used to compare groups in paper I and II as the method does not require the assumption of normal distribution.

## 5 Summary of papers

### Paper I

The aim of the study was to use bioaerosol samples from the personal breathing zone of workers during crab processing to develop and validate a sensitive antibody-based immunoassay for the detection and quantification of the shellfish allergen tropomyosin.

The sampling strategy for collecting bioaerosols samples from the crab processing plants was to collect samples from all the major work stations of the processing line. Through these samples we wished identify the exposure levels of different central components from crab processing in the plant. Interviews with the management and identification of all major work tasks and the number of workers at each area formed the basis for selection of 12 workers at each of the three king crab plants and one edible crab plant who wore the sampling equipment. These 12 workers carried sampling equipment throughout their workday and their work task was registered. Each worker carried one sampling pump connected to a filter (described in section 4.4.1) that was analysed for tropomyosin. The air flow through the sampling pumps and time (minutes) the samplers had run was used to calculate the amount of air that had gone through the filter. Samples were frozen and transported to the Department of Medical Biology at UiT the Arctic University of Norway where they were kept at -20 °C until extraction. The filters were extracted in 1.0 mL PBS with 0.5 % Tween 20 and NaN<sub>3</sub> for conservation, transferred to 1mL mini Eppendorf tubes with bovine serum albumin (BSA) and frozen at -20°C. The samples were transported to co-operators at the Centre for Biodiscovery and Molecular Development of Therapeutics at James Cook University, Australia. Here, anti-tropomyosin antibody was generated in rabbits against tropomyosins from the muscle mass of four different crustacean species (black tiger prawn, Vannamei prawn, Banana prawn and School prawn) and purified using an immune-affinity column. Recombinant tropomyosin from black tiger prawn, Vannamei prawn, Banana prawn and School prawn was expressed in E. Coli, purified and used as an allergen standard for the sandwich ELISA. Limit of detection for the developed sandwich ELISA was 60 picograms/m<sup>3</sup> and limit of quantitation 100 picogram/m<sup>3</sup>.

The method for collecting and analysing tropomyosin in this paper had a high sensitivity and specificity, and can be adapted for the detection of other aerosolised food allergens, assisting in identification of high-risk allergen exposure areas in the food processing industry.

## Paper II

The aim of the study was to find determinants of important exposure agents and personal exposure levels to central components in bioaerosols produced during the processing of king crab and edible crab in Norwegian crab processing plants. And through this to suggest preventive measures to reduce the occupational exposure to components that may cause occupational health problems

Bioaerosol samples were collected from three king crab plants and one edible crab plant. The samples were analysed for tropomyosin, total protein, endotoxin, trypsin, and NAGase. Each worker carried a backpack with three sampling pumps, each connected to one filter cassette (see section 4.4). The edible crab processing generated higher levels than king crab processing in protein ( $GM = 12.9$  vs  $5.1 \mu\text{g}/\text{m}^3$ ) and tropomyosin ( $GM = 45.4$  vs  $2.4 \text{ ng}/\text{m}^3$ ) measurements. However, king crab processing generated higher levels than edible crab processing in endotoxin levels ( $GM = 110$  vs  $72 \text{ EU}/\text{m}^3$ ). Tropomyosin levels were highest during raw king crab processing with  $GM = 9.6$  vs  $2.5 \text{ ng}/\text{m}^3$  during cooked processing. Conversely, edible crab tropomyosin levels were highest during cooked processing with  $GM = 45.4$  vs  $8.7 \text{ ng}/\text{m}^3$  during raw processing. In the edible crab plant, NAGase levels were highest during raw processing with  $GM = 853$  vs  $422 \text{ pmol 4-methylumbelliferon (MU)}/\text{m}^3$  during cooked processing. Trypsin activity was found to be highest during raw processing in both king crab and edible crab plants. When comparing the king crab plants, significant differences were found between the three plants in levels of both tropomyosin and total protein suggesting a plant effect.

There are several important factors affecting the exposure to bioaerosols in both raw and cooked processing of king crab and edible crab. It is necessary to look at the effect preventive measures could have at each separate plant. Important areas are the layout of the processing line, shielding, ventilation, equipment and personal protection of workers to reduce the bioaerosol exposure.

We concluded that Norwegian crab processing workers are exposed to airborne proteins, tropomyosin, endotoxins, trypsin, and NAGase in their breathing zone. Levels vary between king crab and edible crab processing and between processing raw and cooked crab. The difference in exposure levels between the three king crab processing plants suggests a plant effect on bioaerosol exposure levels. Preventive measures need to be taken at each of the plants to reduce the production and dispersion of bioaerosols.

## Paper III

The aim of this study was to examine the respiratory health status of crab processing workers in land based processing plants compared to a control group of workers not working in the seafood industry. Self-reported respiratory symptoms and diagnoses, spirometric results and host-associated factors were analysed.

In a cross sectional study design we compared the respiratory health in two types of crab processing workers to a control group. The study included 148 king crab workers, 70 edible crab workers, and 215 municipal employees who had never worked in the seafood industry. Workers answered a questionnaire containing questions on age, gender, smoking habits, asthma, allergies in addition to respiratory symptoms in the last 12 months. To measure the workers' lung function, spirometry was performed once during the work shift. Predicted lung function values were based on the equations proposed by Langhammer et al. (142) in a healthy, non-smoking, Norwegian adult population. Self reported respiratory symptoms were more common among crab processing workers compared to controls, and more common among king crab workers compared to edible crab workers. There was no significant difference between crab processing workers and controls in lung function results. King crab workers had a higher prevalence of reduced FVC and FEV1/FVC below the 5<sup>th</sup> percentile of predicted values compared to edible crab workers. Self reported doctor diagnosed asthma prevalence was highest in the control group, and significantly higher than among edible crab workers.

We concluded that crab processing workers reported a higher prevalence of respiratory symptoms, but this was not reflected in impaired lung function values or asthma diagnose. Based on the lower prevalence of asthma and allergies, and a higher prevalence of respiratory symptoms among crab processing workers compared to controls, we suggest the presence of a healthy worker effect among crab processing workers in Norway.

## Paper IV

The objective of this work was to examine the sensitisation status to crab among crab processing workers through IgE testing of blood and skin prick testing, and to find the IgE-binding diversity and possible determinants of allergic sensitisation through immunoblotting.

Blood samples were collected from 113 king crab workers and 78 edible crab workers, and analysed for specific IgE to crab. Immunoblots were performed on blood samples from the 10 king crab workers and 10 edible crab workers with the highest specific IgE to crab. Skin prick tests were performed on 40 king crab workers and 83 edible crab workers. Workers also answered a questionnaire about their health.

The four extracts of king crab and edible crab for immunoblots and SPT were made at the Department of Medical Biology at UiT the Arctic University of Norway. The extracts; raw meat, cooked meat, raw shell and raw intestines were frozen until used. King crab workers were tested on king crab extracts, edible crab workers on edible crab extracts. Immunoblots were performed by co-operators at the Centre for Biodiscovery and Molecular Development of Therapeutics at James Cook University, Australia. They examined IgE antibody binding patterns to allergenic proteins in the different crab extracts. Allergograms were generated to compare the IgE antibody binding patterns. SDS-PAGE gel bands were excised and analysed by mass spectrometric analyses.

Specific IgE to crab was established in 8.9 % of king crab workers and 12.2 % of edible crab workers. Positive SPT to one or several components of the crab was established in 17.5 % of king crab workers and 18.1 % of edible crab workers. Both SPT positive and positive specific IgE to crab was established in 12.5 % of king crab workers and 9.6 % of edible crab workers. Edible crab workers had a significantly higher prevalence of SPT positive reactions to shell and cooked crab compared to king crab workers. Most SPT-positive workers reacted to cooked crabmeat extracts, either alone or in combination with other extracts. Atopy was associated with positive SPT, specific IgE to crab, self-reported asthma and allergy. Self-reported respiratory symptoms were associated with self-reported allergy.

Immunoblotting showed more frequent IgE binding for higher molecular weight proteins compared to low molecular weight proteins. Differential IgE binding to crab proteins were observed among the different crab extracts. Cooking of the crab meat resulted in altered IgE binding patterns in the cooked meat extract as compared to raw extract. Arginine kinase was predominantly found in the raw king and edible crab extracts. Tropomyosin was however

found more frequently in the cooked meat extracts. Both tropomyosin and arginine kinase was found in king crab and edible crab shell extracts. Enolase and hemocyanin were in intestine extracts. Enolase was also identified in raw meat extracts. The workers with elevated specific IgE that were tested on immunoblots showed IgE binding to crab allergens. Several workers who reacted to only some of the SPT extracts show IgE binding to all four extracts in the allergograms.

We concluded that many crab processing workers are sensitised to the crab they are processing and thus have increased risk of developing asthma and allergy to crab. Several components in both raw and cooked meat, intestines and shell are sensitising agents and workers have differentiating IgE binding to crab proteins. Two new occupational allergens for crab, enolase and hemocyanin, were identified in both king crab and edible crab extracts. Still, there are several unidentified sensitising allergens that need to be identified to confirm and understand variations in allergenic sensitisation among crab processing workers.

## 6 Discussion

The symptoms reported from seafood industry workers may arise from inhalation of or contact with various exposure agents in their work environment. At the start of this study, it was known that workers processing several types of crustaceans such as shrimp and snow crab, were exposed to bioaerosols containing proteins, including allergens, and endotoxins (Table 1). An increased prevalence of asthma, respiratory symptoms and IgE sensitisation to crustaceans (Table 2) had also been found among these species. We thus set out to explore the occupational exposure to bioaerosols in Norwegian crab processing plants, and the crab processing workers' prevalence of asthma, respiratory symptoms and IgE sensitisation.

### 6.1 Methodology

#### 6.1.1 Study design

This was a cross-sectional study on two crab processing worker populations, king crab workers located in Finnmark county, and edible crab workers located in Sør-Trøndelag county. Crab processing workers were selected on basis of their current employment in the crab processing plant. Some of the crab processing workers were seasonal workers from other countries, mostly Eastern Europe, who arrived for the start of the crab season and moved on at the end of the season. Cultural differences between Norwegian and foreign workers may cause differences in focus on health problems or how to relate to them.

The control group was selected on basis of their current and previous work. Previous work in any type of seafood industry was an exclusion criterion. Few other factories or similar workplaces were available in the geographical areas of the crab production plants so municipal workers were chosen as a control group. By choosing a control group of workers, both groups include subjects within working age that are healthy enough to work. The data from these workers were collected in a previous study using similar data collection tools (see section 4.2.3). Ideally the data should have been collected at the same time for both groups, but financial and time limits prohibited the collection of data from a new control group.

Some essential challenges in data collection on crab processing plants were geographical area and production predictability. Finnmark county is large and with limited access. Due to fear of over fishing, the Norwegian government stopped the king crab fishing season at the beginning of the fishing season 2010/2011. This stopped us from acquiring any data this year. When the new concessions were given out the following year, the priority was given to local

small fishing boats. This made king crab processing less predictable as there were less crab and therefore also fewer processing plants that received king crab. Whether processing plants would receive crab for processing during our planned data collection period was uncertain. Data collection was performed over three or four days at each plant. Only one king crab processing plant had production on all days of data collection. Because the rest of the plants had limited production during our visit, it was not possible to perform repeated measurement of exposure and lung function through a work week, as the workers were not exposed every day. The restrictions in fishing that shortened the season and caused unpredictability for workers, management decisions in who to re-hire and other unspecified reasons caused some turnover of workers from one season to the next. This turnover of workers did not allow for a follow-up of a cohort of workers through consecutive crab processing seasons.

A cross-sectional study measures prevalence and not incidence of findings. The cross-sectional design is less suitable to study exposure-response relations, but it is not impossible and has been done in other studies (40). Our findings from the crab industry still contribute to the knowledge of occupational disease and allergic reactions. It is the first study to describe the exposure in edible crab processing, and the first to analyse NAGase and trypsin activity in crab processing. Moreover, this study used prevalence of health parameters as a risk estimate to describe the workers present situation.

**Confounders** are likely to be present. When collecting data in work places and comparing the exposed workers with a control group that is not perfectly matched in all areas but the area of interest (comparing “like with like”), adjusting for potential confounders may increase validity (161). Internal confounders within a population also need to be taken into consideration, such as smoking may effect the report of respiratory symptoms. Differences between and within the groups were identified through the questionnaire (Appendix C and D) and were used in statistical analyses to adjust for relevant confounders (162). In lung function measurements (paper III), predicted values of FEV<sub>1</sub> and FVC was calculated for each worker based on age, gender and height (142) to compare the lung function parameters between the exposed workers and controls. Through good preparatory work and adjusting for relevant confounders we will reduce the chance for unmeasured confounders.

**Selection bias** based on the volunteer participation in both exposed workers and controls is a possible cause for overestimation of health problems if e.g. those who experience symptoms are more motivated to join than those who do not experience symptoms (161). Or possibly

those who experience health problems do not wish to participate in case they are recommended to stop working in their current job. Among the control group the number of workers who were invited was not known (see section 4.2.3). This may cause an over or under estimation of the prevalence of health problems in the control group. This was not likely to be a major problem in the exposed worker group where almost all workers (90 % of king crab workers and 93 % of edible crab workers) participated. Through personal communication with both workers and employers we were informed that workers had left the crab processing plants due to health problems (13, 163). A selection bias of healthy workers may cause an underestimation of the effect of working in the crab processing industry as the workers who become ill leave for work without the offending exposure and are not included in the cross-sectional study design (12).

**Generalizability**; whether the sample population is representative for other populations, or if the observed associations can be applied to other populations, is central in most studies (161). Future research will compare their results with previously published research in the same area. The crab processing workers in this study may not be entirely representative for crab processing workers everywhere, for instance in size of processing plants, duration of processing season and exposure levels. However, the physiological associations between bioaerosol exposure components of the bioaerosols and development of sensitisation or respiratory symptoms are likely to be applicable in other crab processing populations.

### 6.1.3 Questionnaire

**Information bias** is a challenge when using questionnaires to collect information (161). The questionnaire used in this study was based on validated questionnaires and had previously been used in other studies from the department on workers in the seafood industry (35, 38-40, 137). Response fatigue, when subjects were tired of answering questions, may affect answers in long questionnaires. The questionnaire used in paper III and IV included 51 questions and so was not long. However, for workers whose native language was not Norwegian, this may take some time. To help with answering the questionnaire, the workers were encouraged to ask any of the research staff during data collection.

**Recall bias** is also possible when workers have to answer questions on past exposures and symptoms (164). People who worry about their health may pay more attention and therefore remember their health problems as worse compared to those who do not worry. Similarly

those with e.g. asthma may focus more on their symptoms than healthy workers and so remember more of past ill health.

**Response bias**, particularly **Social desirability bias** (165) is most common in studies that involves self reporting, such as e.g. smoking (166). The workers answer questions in a way they believe will be viewed favourably even though it may not be entirely truthful or accurate. Insufficient benefits for people who are unemployed or lack of compensation for occupational disease may cause underreporting of health problems for both Norwegian and foreign workers. Particularly foreign workers may be afraid they may lose their job if they complain or get sick, and so they may underreport health problems.

#### 6.1.4 Physiological tests

All blood samples were collected by the same researcher and analysed at the Department of Laboratory Medicine at the University Hospital of North Norway. Skin prick tests were performed by three doctors with the same training. Spirometry measurements were performed by a different researcher in the control group than in the crab processing workers because the data for the control group was collected during a previous study in the seafood industry (see section 4.2.3). However, the senior researchers ensured the training and instructions to those performing spirometry measurements in both controls and exposed workers was the same.

#### 6.1.5 Exposure assessments

The basis for differentiation between the three groups of workers in the study was their exposure; king crab, edible crab and non-exposed workers. In paper III, king crab and edible crab workers were combined in one category for some of the statistical analyses. In processing plants the most significant separation of work tasks were between raw and cooked crab processing. In the king crab processing plants, not all workers were stationed in only one area, such as truck drivers and cleaners. They worked in both raw and cooked processing areas and were exposed to bioaerosols containing both raw and cooked crab particulates. The exposure groups in king crab processing plants were therefore categorised as raw processing, cooked processing and overlapping work tasks. In addition to differences between exposure groups, individual differences between workers performing the same work task may produce different levels of bioaerosols (167). However, since the number of workers who wore sampling equipment were not large enough to perform analyses on individual differences, dividing the crab processing workers into raw, cooked and overlapping processing groups

where workers who wore sampling equipment were representatives for their work tasks seem to be the best grouping.

**Laboratory analyses** of each component was performed as described in section 4.4. In paper I, we describe the development and validation of an immunoassay to detect and quantify aerosolised tropomyosin. Previous studies have used serum IgE antibodies from shellfish-sensitised individuals to detect airborne allergens through inhibition ELISA setup (21, 92). Also tandem mass spectrometry (17, 147) has been used to measure allergens in occupational settings. The use of a recombinant protein as standard and purified natural allergen to generate the capture antibody for increased sensitivity and specificity has not been done before. This is a novel method and so has not directly been compared to other methods of quantifying tropomyosin. However, it has a high specificity to crustacean tropomyosin with no non-specific binding. The method did recognise house dust mite tropomyosin, but as the crab processing plants are wet work environments that are often cleaned, the results are not likely to be affected by house dust mite. The immunoassay developed had a detection limit of 60 pg/m<sup>3</sup>. Other methods, such as mass spectrometry, have a lower limit of detection at 0.2 nmol/L for tropomyosin (17). However, all samples collected in the crab processing plants were over the LOD at 60 pg/m<sup>3</sup> so a lower LOD is probably not necessary in this industry. The time and cost benefits of using the immunoassay for analysing multiple samples, makes it a good method for quantifying tropomyosin in bioaerosol samples from crab processing plants.

The presence of trypsin in bioaerosols from seafood processing plants has not previously been shown. Zymography is a sensitive technique allowing the assessment of very low levels of protease activity based on a sodium dodecyl sulfate polyacrylamide (SDS PAGE) gel electrophoresis, with the addition of a protease substrate (eg. gelatin) in the gel (168, 169). Proteases are visualised as clear (unstained) bands where the substrates has been digested and transparency of the gel band is measured and compared to a standard curve of known protease content, present on the same gel. When zymography gels were used with individual standard curves of porcine trypsin, the enzyme activity from the bioaerosol samples could be measured and the results presented on a semi-quantitative scale as shown in paper II.

Total protein, endotoxin and NAGase analyses are established methods with known strengths and weaknesses (158, 170, 171). These have been previously used in occupational exposure studies, including measurements of total protein and endotoxin in studies in the seafood industry.

The results presented in paper I and paper II were 8 hour time weighted averages from the workers' breathing zone. Time weighted average is the most commonly used metric in occupational studies. However, by using time-weighted averages information of variations throughout the workday and any peak exposures are lost. By identifying peak exposures, it is possible to find specific work tasks that produce bioaerosols more efficiently during a workday where workers perform several different work tasks. Separate studies focusing on peak exposures or limiting averages without peaks has been suggested to assess data (172). By collecting the bioaerosols on a filter, it is possible to analyse the bioaerosol components and calculate the average exposure levels of each component. It is also possible to analyse for several components. The combined exposure in bioaerosols, not just single components, are important as the combination of components may have a synergistic effect on the workers' response (37, 172). The use of personal exposure monitoring may be challenging because it requires many measurements and proper equipment. However, it does give the most accurate and representative assessment of the exposure if the workers carrying the equipment wear it properly (173).

## 6.2 Discussion of main findings

### 6.2.1 Occupational exposure

At the start of this study it was known that particulates, proteins including allergens, and endotoxins are present during processing of crustaceans (Table 1). The allergenic proteins present in air-borne particulate matter cause allergic sensitisation in seafood processing workers (61, 149) and have previously been linked to occupational asthma and allergy (7, 9, 16, 25, 30, 108). One major allergen in shellfish is the heat stable allergen tropomyosin that has been identified as a good predictor of shellfish allergy (146, 174, 175). We set out to explore the exposure levels of total protein, tropomyosin, trypsin, endotoxin and NAGase in the personal breathing zone of workers processing raw and cooked king crab and edible crab.

Air samples from the personal breathing zone of crab processing workers were collected during processing of king crab and edible crab and used for analyses included in paper I and II. Few studies have analysed the tropomyosin content in bioaerosols in the king crab industry, and none in the edible crab industry. Neither has NAGase nor trypsin previously been analysed in bioaerosols from the crab processing industry. In this study, we showed that both king crab and edible crab processing workers are exposed to bioaerosols in their personal breathing zone, and that these bioaerosols contain all components we analysed for.

Determining the content of the air inhaled by processing workers will add to the complete knowledge of work place exposures in the seafood processing industry. It can be used to make recommendations to exposed processing workers not only in the Norwegian crab processing industry, but to other countries and other types of seafood processing workers. By gaining knowledge and understanding of exposure and health outcomes, it may be possible to find key determinants of exposure levels and early signs to detect health problems. This would be useful in a new industry where risks are not known, but also in established industries where the focus of exposure and development of health problems has not been a focus area for management, research and medical professionals. A main focus of occupational health services and medical professionals is to prevent work-related ill-health. To be able to do this, it is necessary to find determinants of exposure and components that are responsible for causing occupational health problems and find the best way to protect the workers from these.

The relation between the prevalence of sensitisation, bronchial hyperresponsiveness or asthma may be more dependent on level of occupational exposure to agents than to individual factors such as atopy and smoking (176, 177). A dose-response relationship was found between protein exposure and self-reported respiratory symptoms and lung function in salmon processing workers (40), and reducing the amount of bioaerosols has been found to decrease health problems (49, 86). This illustrates the importance of reducing the exposure levels to bioaerosols. In our study, the levels of total protein and tropomyosin were highest during edible crab processing, while levels of trypsin and endotoxin were highest during king crab processing. Cooked crab processing generated higher levels of tropomyosin than raw crab processing which is in accordance with other studies (178). Important steps to reduce the exposure to tropomyosin would be removing or containing cooking steam and minimizing the manual handling of cooked crab.

In air samples from the edible crab industry, we detected NAGase enzymes, an important enzyme in chitin digestion (76, 79). NAGase may cause an immunological response in the workers when it is inhaled and is linked to sensitisation and asthma (77, 179) as well as ODTs (180, 181). The levels of NAGase measured during edible crab processing was higher than levels found in Danish homes during autumn (157), but lower than occupational exposure measurements in greenhouses (182). Levels of NAGase have been found to decrease with increasing relative humidity (183) which may cause levels to decrease in crab processing plants where the work environment is very wet. However, the NAGase may play a role in the

complete bioaerosol composition and development of health problems in crab processing industries.

Endotoxins are known to cause both allergic and non-allergic respiratory diseases, lung function impairment and ODTs, particularly among farmers (1, 180, 181, 184). The “no adverse health effect” to endotoxins suggested at 90 EU/m<sup>3</sup> (185) was exceeded in 4 of 7 samples from the king crab industry and 2 of 8 samples in the edible crab industry, all during raw processing. The mean level of endotoxin found in Alaskan snow crab processing workers was 15.6 EU/m<sup>3</sup> in the respirable fraction (4) which is lower than both king and edible crab workers (6285.5 and 72 EU/m<sup>3</sup> respectively) in our study. The range of exposure has been high in both previous studies with measurements up to 949 EU/m<sup>3</sup> in the Alaskan study, and in our study where a measurement in raw king crab processing found 24000 EU/m<sup>3</sup>. At these levels, an effect of the endotoxin exposure such as flu-like symptoms or ODTs may be expected. Because of these large variations in exposure levels, some of which were very high, collection of information on work tasks linked to measurements is needed to gain knowledge of determinants of exposure and to find ways to reduce it.

Trypsin activity was higher in samples from raw crab processing compared to cooked processing. Trypsin has been found to activate inflammatory signalling in cell model studies in both skin cells and airway epithelial cells (36, 37). The presence of both trypsin and endotoxin (LPS) have been found to have a synergistic effect on inflammatory signalling in cell models (37, 186, 187), illustrating the importance of considering the combined exposure at the work place. The presence of proteases in work environments is best known from the detergent industry (188-190). However, most analytical approaches to enzymes in the work environment have not quantified the low levels from bioaerosol samples. To our knowledge, paper II is the first published paper where the presence and semi quantitative levels of trypsin activity have been shown in the seafood processing industry. This is an important component in bioaerosol exposure that needs to be taken into consideration when assessing work place exposures.

Differences in exposure levels to allergens between work areas and exposure groups have been reported (17, 21, 92, 191). In our study there were differences in exposure levels between king crab and edible crab processing, raw and cooked crab processing, as well as differences between the king crab plants. The “plant effect” (see definition of concepts) found between the different king crab processing plants show that the levels of exposure varies between different plants. The plant layout, placement of the processing line, processing

techniques and ventilation may be central to bioaerosol exposure levels and distribution. It is of interest to identify which parameters have the greatest impact on the production and distribution of bioaerosols to implement protective measures in these areas. This study shows the importance of work place adjustments to minimize the exposure of bioaerosols to processing workers in order to prevent development of occupational health problems.

### 6.2.3 Occupational health

At the start of this study we knew that crab processing workers in Alaska and Canada have a high risk of developing sensitisation to crab, occupational allergy and asthma (9, 25, 29-32). Upper respiratory symptoms such as rhinitis and hay fever often precede the development of asthma and has been found among seafood processing workers (121, 192-195). Tropomyosin and arginine kinase have been identified as major allergenic proteins in crustaceans (17, 28, 61, 147, 196). More workers are sensitised to heated tropomyosin than to raw (64-66). We set out to explore the prevalence of respiratory health and sensitisation among workers processing king crab and edible crab. We also wished to determine IgE-binding diversity and components to king crab and edible crab among processing workers. Health examinations and a questionnaire study was conducted on king crab workers, edible crab workers and non-exposed controls and the results are presented in paper III and IV.

Crab processing workers have a higher prevalence of some respiratory symptoms compared to the non-exposed controls, but no statistically significant difference was found between exposed workers and controls in lung function parameters from spirometry measurements. However, when comparing the two populations of crab processing workers, king crab workers had a higher prevalence of reduced FVC, FEV<sub>1</sub>/FVC below the 5<sup>th</sup> percentile of predicted values, and a higher prevalence of shortness of breath than edible crab workers. A study on king crab processing workers in the USA reported similar findings of respiratory symptoms as the king crab workers in our study (94), but higher than the edible crab workers. The increased prevalence of respiratory symptoms among crab processing workers compared to non-exposed controls support previous findings that seafood processing workers are at risk of developing respiratory symptoms (3, 20, 29, 109). Despite the increased levels of protein and tropomyosin in edible crab processing compared to king crab processing, the prevalence of respiratory symptoms were lower among edible crab workers. One of the reasons for this may be that the increased levels of trypsin or endotoxin found in king crab processing have a larger effect on the respiratory symptoms than allergens. Enzymes and endotoxins may have an irritative effect on the airways that have an immediate effect causing respiratory symptoms,

while the development of respiratory symptoms as an allergic reaction requires repeated exposures and sensitisation. However, attention from management in the edible crab processing plant on occupational health and access to respiratory protection could be a central reason for this difference. It is important in all occupational settings for upper management to show interest and commitment in preventing ill health (197). By ensuring employee participation and training, workers may be more likely to follow implementations and comply with the use of protective measures such as shielding of work tasks and personal protective equipment. By keeping a focus on protecting the workers, they may also learn to recognise respiratory symptoms at an early stage and prevent the development of more serious illness such as asthma.

The prevalence of SPT positive workers were similar between king crab and edible crab workers with SPT positive results in 17.5 % of king crab and 18.1 % of edible crab workers to in house extracts of raw meat, cooked meat, raw shell and raw intestines. Our findings are lower than previous studies among snow crab processing workers (29, 30, 108). This may be related to differences in processing, exposure, or use of personal protective equipment, or the SPT extracts used. Since there are no commercially available extracts of raw meat, shell and intestines of king crab and edible crab, they had to be made for this study. When extracts are made independently for each study, any differences between laboratories in the production would not be traceable. The workers will however be tested on the product they are exposed to at work, and to which they may be sensitised. Previous studies have indicated that heating shellfish increases the antibody reactivity to tropomyosin (64, 146, 198). Similar results was found among the crab processing workers in our study where most SPT positive workers reacted to the cooked crab meat extract, either alone or in combination with the other raw extracts.

The immunoblots performed on the 10 king crab and 10 edible crab workers with the highest specific IgE to crab in the Phadia test showed IgE binding to several proteins in all the four crab extracts. A comparison of the allergograms of IgE reactivity between identical proteins in raw and cooked meat indicates a higher number of IgE binding proteins in the raw crabmeat compared to the cooked crabmeat in both types of crab. The king crab processing was mainly performed on raw crab so most workers would be handling raw crab while in edible crab processing was mainly performed on cooked crab so most workers would be handling cooked crab. This is reflected in the allergogram where the king crab processing workers had most high IgE binding to the raw meat while edible crab processing workers had

most high IgE binding to the cooked meat. This indicates that the different processing methods as well as isolated crab sections cause altered IgE binding to the various allergens. This is the first time a direct comparative analysis investigating differential binding analysis between raw meat, cooked meat, intestine and crab shell extract has been conducted. When comparing the results of the SPT to the allergograms, the two methods differs in some workers. For instance, king crab worker number 2 from the allergogram in paper IV did not have a positive reaction in the SPT, but did show IgE binding in the immunoblot. Similar results were identified in edible crab worker number 2 and 3 from the allergogram in paper IV where the workers did not have positive SPT results or show IgE binding to cooked crab meat, but did have elevated specific IgE to the Phadia test that is made from cooked edible crab meat. Since the Phadia test is often the only available method for medical professionals to examine patients for suspected sensitisation or allergy, it is important to be aware of its limitations.

Tropomyosin and arginine kinase were identified in all the king crab and edible crab extracts. Additionally hemocyanin, an oxygen-transport protein in crustacean hemolymph, was identified in both king crab and edible crab intestine extracts. Hemocyanin is an ingestion related allergen in crab roe (152) and shrimp as well as an inhalant cockroach allergen (151). Moreover, enolase which is an enzyme in the glycolysis, has been considered a putative novel shellfish allergen (153), was identified in raw meat and intestine extracts in both king crab and edible crab. Enolase in fish has been found to be a heat sensitive allergen (199) and was only identified in raw crab components in our study. Hemocyanin was also only identified in raw intestines despite being heat stable (200) suggesting the limited distribution of this allergen in the crab to the carapace. These findings suggests that hemocyanin and enolase are more important as allergens in the work environment than in food consumption.

The route of exposure in the general population is through ingestion of cooked crab, while crab processing workers are mainly exposed through the respiratory system, and to a lesser degree, through the skin. The commercial allergy tests based primarily on food exposure may not be able to detect the allergy developed by workers primarily exposed through processing the crustaceans. The levels of exposure in occupational settings are also very different from consumers. While people mostly eat crab a few times during the season, a processing worker will be exposed to much higher levels through the whole workday, which can last 12 hours. A crab processing worker will also be handling the shell, intestines and raw crab, and so will be likely to develop sensitisation to these components as well as the finished product.

Additionally, the consumer will not be exposed to the other components that combines with the crab to make the complete occupational bioaerosol exposure such as endotoxins and enzymes. Because of this, it is important to focus on the total occupational exposure burden and not just the general allergy test available from their general practitioner for suspicion of crustacean allergy. All these other factors need to be taken into consideration when a crab processing worker develops health problems. This study mainly focused on sensitisation to tropomyosin and arginine kinase. However, other sensitising allergens were observed through immunoblots and two new allergens – hemocyanin and enolase – were identified, and they were identified in extracts consumers are not usually exposed to. These novel crab allergens may play a role in the inhalational sensitisation of processing workers handling crab. A detailed proteomic analysis of all the IgE binding proteins in king and edible crab may assist in identification of yet unknown airborne allergens responsible for occupational sensitisation to crab. Training and focus in crab processing plants as well as in the occupational health service and with general practitioners of these differences between occupational exposure and the general exposure in consumers may result in proper investigation and early detection of occupational health problems.

There was no significant association between sensitisation to crab and respiratory symptoms, which indicates that even if workers are sensitised to crab they have not necessarily developed occupational asthma or allergy. Another reason for the lack of association may be the study design. The cross-sectional design of the study may cause an underestimation of occupational health problems in both king crab and edible crab processing plants as the study does not include those workers who left their work due to the development of respiratory symptoms or asthma. This may cause an underestimation of the true prevalence of health problems among workers in jobs with high risk of occupational disease. Through personal communication with the workers and the management at the processing plants, we were informed that at the start of each season, there were some workers that did not return because of health problems. This causes a healthy worker effect in cross-sectional studies which results in an underestimation of the effect working in the crab processing industry has on the exposed workers' health. There are several observations that strengthen the assumption of a healthy worker effect in this study. The short duration of employment (king crab workers median = 1.6 years, edible crab workers median = 1.5 years) may be caused by the workers having to leave due to occupational health problems. The lower prevalence of self reported asthma and family history of asthma and allergy among crab processing workers compared to the control group

further support the finding of a healthy worker effect. We also found lower prevalence of sensitisation, asthma and respiratory symptoms than other studies in the crab processing industry. Our findings may indicate that workers are sensitised to crab, but they have not yet developed respiratory symptoms that may lead to occupational asthma or allergy. Sensitisation is the first step towards potentially developing allergic asthma, and upper respiratory symptoms would follow sensitisation and precede the development of more serious health problems such as asthma and allergy (121, 192). It may be, that when sensitised workers develop respiratory symptoms, and these symptoms become uncomfortable, they leave the processing plants. This is likely to have happened in the plants in our study. If this is the case, the workers with occupational asthma and allergy would not be included due to the healthy worker effect.

### 6.2.3 Implications of our findings

In this study, we have showed that crab processing workers are exposed to bioaerosols in their breathing zone that contain several components which may cause occupational health problems. Workers were sensitised to the crab they were processing and respiratory symptoms were also reported. The crab processing workers did not have an increased prevalence of asthma or allergy compared to non-exposed controls. In fact, they reported less asthma and allergy than the controls. This, along with other findings suggests a healthy worker effect that causes an underestimation of work-related health effects.

The sensitisation to crab among the crab processing workers suggest they are at risk of developing occupationally related health problems. There was no increased prevalence of asthma among the crab processing workers, but around 17 % of the workers were sensitised to crab, and the increased prevalence of self reported respiratory symptoms suggest that several of the workers may have begun developing health problems that may lead to an occupational asthma or allergy. It is important to start measures to reduce the relevant exposure and prevent workers developing health problems.

It is of interest to identify which parameters have the greatest impact on the production and distribution of bioaerosols to implement protective measures in these areas. When starting the implementation of measures to ensure the exposure to bioaerosols is as low as possible, general measures to the layout of the plant and processing line is the first step. Information on the plant effect and which areas can be changed to better the layout of the processing line is important. An important section of the plant effect could be choosing where to place moveable sections of the processing line in relation to ventilation, doors, freezers and areas with high activity of truck driving or cleaning. Placement of the ventilation system (inlet and outlet) when setting up the processing line can optimise the removal of bioaerosols and reduce the bioaerosol exposure levels to workers. Point ventilation in areas where most of the bioaerosols are produced, such as over cooking vats or by the de-gilling area, would further facilitate the removal of bioaerosols where crab processing workers are located. Measures like this has improved the health of workers in other studies (49, 86). To reduce the dispersion of bioaerosols some work tasks may be placed in separate rooms (such as cooking rooms or cleaning rooms for the vats) to minimise the exposure to as few workers as possible. Substituting the use of water hose or minimising the pressure of the water spray will reduce the production of aerosols. So will cleaning floors by using a rubber wiper instead of spraying with a water hose. If some work tasks are completely or partly automated, it may be possible



Figure 14 Tumbler for separating edible crab shell and meat

to enclose them. One example of where this may be possible in the edible crab industry is one station where they rinse small pieces of meat from small bits of shell with water jets and sieves (Figure 14). The tumbler where the water jets eject water could be enclosed, and only the end of the sieves accessible all the time for the workers to remove the shell and meat. An important contribution to bioaerosol production in both king crab and edible crab processing is the cleaning/de-gilling stations with rotating brushes where parts of the crab (such as dirt on the shell, gills or small pieces of meat) are removed (Figure 2, 3 and 6). Optimising these work tasks may reduce the amount of crab becoming aerosolised. Automation of processing, such as de-gilling the clusters, would make it possible to enclose the process which would likely greatly reduce the bioaerosol production. It is important to include the management, the occupational health service and the workers themselves in the discussions of new implementations is important to ensure the measures are affordable, possible to implement, and that they will be used.

If effective general measures are not possible, the use of personal protective equipment such as respirators is possible. If workers are given respirators, it is important to ensure the respirators protect the workers from the exposure. Performing fit testing to ensure that each worker use the mask that best fit them, attaching the correct filter type, training the worker in wearing, cleaning and proper maintenance and storage is necessary for optimal protection. If respiratory symptoms occur, early intervention is important to ensure the workers stay healthy. The duration of exposure after symptoms occur is important for their recovery prognosis (123). Ensuring proper training for all workers and creating awareness of the challenges in crab processing among management and workers can prevent development of disease. Information on risk is central as not all workers may attribute delayed reactions such as breathlessness they may experience at night to the exposure at work several hours earlier. Occupational health services and medical doctors also need to be informed of the risk attributed to working in the seafood industry so they can recognise the symptoms and provide the best possible advice and help. Through knowledge, we may increase compliance in use of preventive measures such as protective equipment, and early warning signs may be caught. Follow-up of workers by the occupational health services' medical staff to ensure an early response if workers develop symptoms can prevent workers from developing asthma and allergy, and they may stay healthy and working for longer.

## 7 Conclusions and future research

### 7.1 Conclusions

The following conclusions can be drawn from this study

- The developed immunoassay for air-borne tropomyosin is a good method for quantifying tropomyosin levels in air samples taken from the breathing zone of crab processing workers.
- Crab processing workers are exposed to bioaerosols containing the major allergen tropomyosin, the enzymes trypsin and NAGase, other proteins and endotoxin in their work environment. Levels of endotoxin and trypsin were highest in king crab processing while total protein fraction and tropomyosin levels were highest in edible crab processing.
- Exposure levels vary between raw and cooked crab processing. Processing cooked crab generates higher levels of tropomyosin than raw crab processing. Processing raw crab generates higher levels of trypsin than cooked crab processing. Exposure levels also vary between king crab and edible crab workers and between the king crab processing plants, suggesting a plant effect.
- Crab processing workers report a higher prevalence of respiratory symptoms than non-exposed controls. These symptoms were not reflected by impaired lung function values or an increased prevalence of asthma diagnosis.
- Workers processing both king crab and edible crab are sensitised to crab. Skin prick tests found that cooked edible crab meat extracts were more potent compared to raw edible crab extracts. No difference was found between king crab extracts.
- Sensitised crab processing workers have IgE reactivity to several proteins in different crab extracts and not all workers are sensitised to the major allergen tropomyosin.
- Tropomyosin, arginine kinase, enolase and hemocyanin are identified as allergens in both king and edible crab sensitised workers.
- Based on a lower prevalence of asthma, allergy and family history of asthma and allergy among crab processing workers compared to controls, an increased prevalence of respiratory symptoms and sensitisation to crab in specific IgE test, skin prick test and immunoblots, we suggest the presence of a healthy worker effect among crab the processing workers in our study.

## 7.2 Future research

Occupational exposure and health among crab processing workers has been studied for over 30 years, yet there are still unsolved questions that require further research.

- Because most studies are cross-sectional, cohort studies focusing on the incidence of health outcomes should be performed as a healthy worker effect may cause an underestimation of the health effects of working with crab processing.
- The time from exposure starts until symptoms occur and the order in which respiratory symptoms occur should be assessed since time from symptoms occur until removal from exposure is important for recovery.
- Since not all crab processing workers are sensitised to the same allergens, identifying the different sensitising agents could improve diagnosis. Improved diagnosis could be used to identify work tasks a sensitised worker should avoid if they are found to have IgE sensitisation to specific components.
- Intervention effect should be studied to find how preventive measures may change the exposure. Identifying which processes generates the different bioaerosols and focus on the effect of changing each of the processes as well as evaluating the effect of an intervention. This way effective measures can be identified and implemented in processing procedures or layouts not only in crab processing plants, but other seafood processing plants or workplaces with similar challenges.
- There is a lack of occupational guidelines for exposure to biologically active exposures.
- Exposure-response relationships to different components should be assessed.
- Studies in the seafood industry have identified both allergic and irritant induced respiratory symptoms. Causal mechanisms for the respiratory symptoms caused by crab processing need to be identified.
- The combined effect of trypsin and endotoxin has been found in cell models. Further studies are needed on the effect of the combined exposures found in the crab processing plants.
- Identifying allergens that elicit IgE sensitisation to develop better commercial tests for occupational sensitisation to crab.

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# **Appendix A**

**Information about the study and informed consent form  
for the control group (Norwegian)**



# **Forespørsel om deltagelse i et forskningsprosjekt i regi av Arbeids- og miljømedisinsk avdeling, Universitetssykehuset Nord-Norge**

## **Kontrollgruppe**

Universitetssykehuset Nord-Norge har tidligere gjennomført en undersøkelse om arbeidsmiljø og helse i fiskeindustrien i Nord-Norge. Et av de viktigste funnene i den forrige undersøkelsen var en økt forekomst av luftveisplager i tilknytning til arbeid og kontakt med råstoffet. Vi er nå i gang med et nytt forskningsprosjekt der hovedformålet er å skaffe ny kunnskap om sammenhenger mellom eksponeringer og luftveisplager i lakseindustrien. Denne kunnskapen er viktig for å kunne forebygge plagene.

I dette prosjektet trenger vi også deltagelse fra arbeidstakere som ikke jobber i fiskeindustrien, som skal fungere som en ”kontrollgruppe” som vi kan sammenligne funn fra lakseindustriarbeidere med. Vi spør deg derfor om du vil svare på spørsmål om arbeidsmiljøet og helsen din. Det er frivillig om du vil være med i undersøkelsen, og du må ikke begrunne hvorfor du eventuelt ikke vil delta. Det vil heller ikke få noen konsekvenser for forholdet til arbeidsplassen eller på annen måte om du ikke vil delta eller trekker deg på et senere tidspunkt. Selv om du bestemmer deg for å delta nå, kan du senere trekke deg når du ønsker, og opplysningene som er samlet inn om deg vil bli slettet hvis du ønsker det.

Prosjektet er finansiert av Helse-Nords forskningsmidler. Undersøkelsen er tilrådd av Personvernombudet for forskning, Norsk samfunnsvitenskapelige datatjeneste AS. Regional komité for medisinsk forskningsetikk, Nord-Norge, har vurdert prosjektet og har ingen innvendinger mot at prosjektet gjennomføres.

Det kan bli aktuelt å gjennomføre en oppfølgingsstudie ved et senere tidspunkt, og vi ønsker derfor å oppbevare de innsamlede opplysningene med personidentifikasjon i inntil 10 år i påvente av en slik undersøkelse. Opplysningene vil bli oppbevart ved en arkivinstitusjon som er godkjent av datatilsynet for oppbevaring av persondata. Ingen data vil være tilgjengelig for andre. Vi spør derfor om ditt samtykke til at opplysningene om deg blir arkivert etter prosjektets avslutning.

Vi har henvendt oss til bedriftshelsetjenesten for å få hjelp til å finne egnede arbeidstakere som kan inngå i en kontrollgruppe. Bedriftshelsetjenesten har mottatt spørreskjemaet og delt det ut til ansatte i din og andres bedrifter. Hvis du velger å delta skal det utfylte spørreskjemaet og samtykke-erklæringen sendes direkte til oss. Ingen fra bedriftshelsetjenesten har adgang til besvarelserne. Prosjektlederne lager en liste der navn og referansenummer kobles, og det er kun de som kjenner din identitet.

Samtykke-erklæringen, der du skriver navnet ditt, vil bli oppbevart adskilt fra spørreskjemaet. Ved senere publisering vil ingen opplysninger kunne føres tilbake til enkeltpersoner eller bedrifter.

Noen av de som besvarer spørreskjemaet vil senere få spørsmål om å delta i enkle medisinske undersøkelser, som lungefunksjonsundersøkelser og allergitester (blodprøver). Disse undersøkelsene vil ikke føre til ubehag utover et stikk i armen. Vi spør derfor om din tillatelse til å kontakte deg med forespørsel om en slik undersøkelse på et senere tidspunkt. Selv om du samtykker til å bli spurt har du likevel mulighet til å la være å samtykke til deltagelse i de medisinske undersøkelsene hvis du får henvendelse om dette.

Du får to kopier av dette brevet. Hvis du velger å delta sender du inn ett underskrevet eksemplar en ferdigfrankert konvolutt. Det andre eksemplaret beholder du selv. Besvart spørreskjema sendes i den andre ferdigfrankerte konvolutten.

Du kan når som helst ta kontakt med prosjektlederne på tlf 77628498 eller 77627463.

Med vennlig hilsen

Lisbeth Aasmoe og Berit Bang (prosjektledere)  
Universitetssykehuset Nord-Norge

Referansenummer:

**Samtykke-erklæring**

Ja

Jeg samtykker i å delta i spørreundersøkelsen



Jeg samtykker i å bli spurt om å delta i en begrenset helseundersøkelse ved et senere tidspunkt



Jeg samtykker i at opplysningene om meg arkiveres etter prosjektets avslutning



---

Dato/Navn (blokkbokstaver)

signatur

## **Appendix B**

Information about the study and informed consent form  
for crab processing workers (English and Norwegian)



# **Request for participation in research project**

## **"Respiratory problems and allergies in the crab industry"**

### **Background and intentions**

This is an inquiry for your participation in a research study where we wish to gather new knowledge on the relation between exposures and respiratory problems in the crab industry. The project started in 2009 and will end in 2012.

The University Hospital of North Norway has earlier conducted research on work environment and health in the fish industry in Northern Norway. One important finding was the increased occurrence of respiratory problems in association with the work. Other studies in Canada and Alaska suggest that the development of respiratory problems and asthma in connection with processing of crab can be a significant problem among production workers in the industry. We also think it is important to bring this information back to the industry, to assist selective for health and safety measures and research on work environment.

### **The content of the study**

We ask you to answer questions about your work environment and your health. This is voluntary, and should you choose not to participate, you do not have to give any reason. If you decide not to participate or withdraw at a later time, this will not affect the relation to your work place. Some of you will be asked to participate in examinations, such as lung function studies and allergy tests at a later time. These examinations will not cause any discomfort other than a needle-prick in the arm.

**The workplace has received the questionnaire and distributed it among the employees. If you choose to participate, you fill out the questionnaire and the consent declaration and return it directly to us, closed in the stamped envelope, enclosed. No one from the work place will have access to your answers.**

Only the project leader has access to the list connecting each name with a serial number on the questionnaire. The answers will be treated in the strictest of confidence. Only the project leader, no one else who handles the questionnaire, will know your identity,

### **Possible advantages and disadvantages**

The medical examinations will not cause any discomfort other than needle-prick in the arm.

### **What will happen to the test and the information about you?**

The tests taken of you and the registered information will only be used as described in the purpose of the study. All information and tests will be treated without names, birth date, or other recognisable information. A code connects you to your data and samples through a list of names. Only authorised personnel involved in the project has access to the name list and can trace the number back to the name.

It may be of interest to implement a follow-up study at a later time. We therefore wish to store the gathered information with person identification for up to 10 years in case of such a study. The information will be stored in an archival institution approved by the Data Inspectorate for

storage of personal data. No data will be accessible for others. It will not be possible to identify you in the results of the study when it is published.

### **Volunteer participation**

Participation in this study is on a volunteer basis. You can at any time, and without naming a reason, withdraw your participation in the study. This will not have any consequences to your relationship with your work place. If you wish to participate, you sign the consent form on the last page of this information pamphlet. If you agree to participate, but at a later time wish to withdraw from the study, or if you have any questions about the study, you can contact Lisbeth Aasmoe on telephone number 77628498.

### **Privacy**

Information that is registered about you is information like name, age, the company you work in, and any health problems, especially in connection with work.

The University Hospital of North Norway with administrative director is responsible for the treatment of your data.

### **Bio bank**

The blood samples taken and the information derived from this material will be stored in a research bio bank at the University Hospital of North Norway. If you agree to participate in the study, you also consent to include the biological material and results from the analyses that are included in the bio bank. The project leader/scientist Lisbeth Aasmoe is in charge of the research bio bank which is planned to last until 2020. After this, the material and all information will be destroyed and deleted by internal guidelines.

### **Right to insight and deletion of information about you and destruction of tests**

If you agree to participate in this study, you have a right to insight in the registered information about you. You also have a right to have any errors in the registered information corrected. If you withdraw from the study, you have a right to have all tests and information about you erased, unless the information is already included in analyses or used in scientific publications.

The study and the bio bank are financed by Extra-funds from Health and Rehabilitation.

### **Insurance**

The participants are insured through the Norwegian patient damage insurance

### **Information on the outcome of the study**

You will be properly informed if we should find anything irregular in your test results, or under the health examination.

With regards

Project leader/scientist Lisbeth Aasmoe  
Department of occupational and environmental medicine  
University hospital in Northern Norway

# **Consent to participate in the study**

I agree to participate in the study

---

(Signed by project participant, date)

repeat name with capital letters

# **Forespørsel om deltagelse i forskningsprosjektet**

## **"Luftveisplager og allergi i krabbeindustrien"**

### **Bakgrunn og hensikt**

Dette er et spørsmål til deg om å delta i en forskningsstudie der vi ønsker å skaffe ny kunnskap om sammenhenger mellom eksponeringer og luftveisplager i krabbeindustrien. Prosjektet starter opp i 2009, og avsluttes i 2012.

Universitetssykehuset Nord-Norge har tidligere gjennomført undersøkelser om arbeidsmiljø og helse i fiskeindustrien i Nord-Norge. Et av de viktigste funnene var en økt forekomst av luftveisplager i tilknytning til arbeid og kontakt med råstoffet. Andre studier fra Canada og Alaska antyder at utvikling av luftveisplager og astma i forbindelse med prosessering av krabbe kan være et betydelig problem hos produksjonsarbeidere i industrien. Vi ser det også som viktig å tilbakeføre denne kunnskapen til næringen og være et utgangspunkt for å peke ut satsingsområder og gjøre prioriteringer innen arbeidsmiljørarbeid.

### **Hva innebærer studien?**

Vi spør deg derfor om du vil svare på spørsmål om arbeidsmiljøet og helsen din. Det er frivillig om du vil være med i undersøkelsen, og du må ikke begrunne hvorfor du eventuelt ikke vil delta. Det vil heller ikke få noen konsekvenser for forholdet til arbeidsplassen eller på annen måte om du ikke vil delta eller trekker deg på et senere tidspunkt.

Vi planlegger å gjøre enkle medisinske undersøkelser, som lungefunksjonsundersøkelser og allergitester (blodprøver), på noen av dere på et seinere tidspunkt. Disse undersøkelsene vil ikke føre til ubehag utover et stikk i armen.

**Bedriften har mottatt spørreskjemaet og delt det ut til alle ansatte. Hvis du velger å delta skal det utfylte spørreskjemaet og samtykke-erklæringen sendes direkte til oss i lukket i en frankert konvolutt som er vedlagt.**

Ingen fra bedriften har adgang til besvarelsene. Prosjektlederne lager en liste der navn og referansenummer kobles. Svarene blir behandlet strengt fortrolig. Det er kun prosjektlederne som kjenner din identitet, ingen andre som handterer spørreskjemaet kjenner din identitet.

### **Mulige fordeler og ulemper**

De medisinske undersøkelsene vil ikke føre til ubehag utover et stikk i armen.

### **Hva skjer med prøvene og informasjonen om deg?**

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg.

Det kan bli aktuelt å gjennomføre en oppfølgingsstudie ved et senere tidspunkt, og vi ønsker derfor å oppbevare de innsamlede opplysningene med personidentifikasjon i inntil 10 år i påvente av en slik undersøkelse. Opplysningene vil bli oppbevart ved en arkivinstitusjon som

er godkjent av datatilsynet for oppbevaring av persondata. Ingen data vil være tilgjengelig for andre. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres

### **Frivillig deltagelse**

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for ditt forhold til arbeidsplassen. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte Du kan når som helst ta kontakt med prosjektleder/forsker Lisbeth Aasmoe på tlf 77628498.

### **Personvern**

Opplysninger som registreres om deg er personopplysninger som navn og alder, i hvilken bedrift du jobber, og eventuelle helseplager i forbindelse med arbeid.

Universitetssykehuset Nord-Norge ved administrerende direktør er databehandlingsansvarlig.

### **Biobank**

Blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Universitetssykehuset Nord-Norge. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Prosjektleder/forsker Lisbeth Aasmoe er ansvarshavende for forskningsbiobanken. Biobanken planlegges å vare til 2020. Etter dette vil materiale og opplysninger bli destruert og slettet etter interne retningslinjer.

### ***Rett til innsyn og sletting av opplysninger om deg og sletting av prøver***

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Studien og biobanken er finansiert med Extra-midler fra Helse og Rehabilitering.

### **Forsikring**

Deltakerne er forsikret gjennom Norsk pasientskadeforsikring

### **Informasjon om utfallet av studien**

Du vil få skriftlig beskjed fra oss dersom vi finner noe uregelmessig i prøvene eller under en eventuell helseundersøkelse.

Med vennlig hilsen

Prosjektleder/forsker Lisbeth Aasmoe  
Arbeids- og miljømedisinsk avdeling  
Universitetssykehuset Nord-Norge

# **Samtykke til deltagelse i studien**

Jeg er villig til å delta i studien

---

(Signert av prosjektdeltaker, dato)

gjenta navn med blokkbokstaver

# **Appendix C**

Questionnaire for the control group (Norwegian)



# ARBEIDSMILJØ OG LUFTVEISPLAGER

Reg.nr

1080

Skjemaet skal leses optisk. Vennligst bruk blå eller sort penn. Du kan ikke bruke komma, bruk blokkbokstaver.

## PERSONALIA

1. Kjønn  Mann  Kvinne

2. Fødselsår (eks 1963) .....

--	--	--

3. Er du norsk eller nordisk statsborger med fast bosted i Norge?

Ja  Nei

4. Har du bodd sammenhengende i Norge de siste fem årene?

Ja  Nei

5. Hvor mange års utdanning har du totalt (inkludert barneskole, ungdomsskole, videregående skole, senere skolegang/studier)

--	--	--

6. Spiser du fisk?

Ja  Nei

7. Hvis ja, hvor ofte spiser du fisk?

		ganger pr uke
		ganger pr mnd

8. Jobber du i lakseindustrien?

Ja  Nei

Hvis nei, gå til spørsmål 15



9. I hvor mange år har du totalt jobbet i lakseindustrien?

--	--	--

10. Hvor i bedriften jobber du – hvilken avdeling?

Ja, mer enn  
halvparten av tida      Ja, mindre enn  
halvparten av tida

Slakteri    
Videreforedling av laks  
(Filetering, porsjonspakkning etc.)    
Administrasjon/kontor    
Annet

11. Hvor i bedriften jobber du – hvilke arbeidsoppgaver har du?

Ja, mer enn  
halvparten av tida      Ja, mindre enn  
halvparten av tida

- |   |                          |                          |
|---|--------------------------|--------------------------|
| Bløgging  | <input type="checkbox"/> | <input type="checkbox"/> |
| Sløyemaskin   | <input type="checkbox"/> | <input type="checkbox"/> |
| Etterrensing  | <input type="checkbox"/> | <input type="checkbox"/> |
| Skjæring/kutting av filet med<br>maskiner               | <input type="checkbox"/> | <input type="checkbox"/> |
| Filetkutting for hånd                                   | <input type="checkbox"/> | <input type="checkbox"/> |
| Røyking av fisk   | <input type="checkbox"/> | <input type="checkbox"/> |
| Vektsortering/kvalitetskontroll av<br>fisk              | <input type="checkbox"/> | <input type="checkbox"/> |
| Kjølerom/kjølelager                                     | <input type="checkbox"/> | <input type="checkbox"/> |
| Fryselager  | <input type="checkbox"/> | <input type="checkbox"/> |
| Håndtering av fiskeavfall                               | <input type="checkbox"/> | <input type="checkbox"/> |
| Produksjon av fiskemat/<br>videreforedling av produkter | <input type="checkbox"/> | <input type="checkbox"/> |
| Teknisk vedlikehold av produk-<br>sjonsmaskiner         | <input type="checkbox"/> | <input type="checkbox"/> |
| Laboratorium  | <input type="checkbox"/> | <input type="checkbox"/> |
| Kontor/administrasjon                                   | <input type="checkbox"/> | <input type="checkbox"/> |
| Annet   | <input type="checkbox"/> | <input type="checkbox"/> |

12. Arbeider du med spyling (f. eks gulv, maskiner) på din arbeidsplass?

Ja, ofte (hver dag)  Ja, iblant  Nei, sjeldent

13. Bruker du maske/munnbind når du jobber?

Alltid  Av og til  Sjeldent/aldri



14. Hva slags aktivitet har du vanligvis hatt i arbeidet ditt siste 12 måneder? (ett kryss)

- Lett fysisk aktivitet, for det meste stillesittende/stående arbeid (f.eks kontorarbeid, filetkutting)   
Arbeid som krever at du beveger deg mye   
Arbeid som krever at du beveger deg og løfter mye   
Tungt fysisk arbeid

**32. Hvordan vurderer du din egen helse sånn i alminnelighet?**

(ett kryss)

- |                         |                          |              |                          |
|-------------------------|--------------------------|--------------|--------------------------|
| Meget god               | <input type="checkbox"/> | Dårlig       | <input type="checkbox"/> |
| God                     | <input type="checkbox"/> | Meget dårlig | <input type="checkbox"/> |
| Verken god eller dårlig | <input type="checkbox"/> |              |                          |

**33. Hvordan synes du at helsen din er sammenlignet med andre på samme alder? (ett kryss)**

- |             |                          |                |                          |
|-------------|--------------------------|----------------|--------------------------|
| Mye bedre   | <input type="checkbox"/> | Litt dårligere | <input type="checkbox"/> |
| Litt bedre  | <input type="checkbox"/> | Mye dårligere  | <input type="checkbox"/> |
| Omtrent lik | <input type="checkbox"/> |                |                          |

**HELSEPLAGER I FORBINDELSE MED ARBEIDET**

Spørsmålene under dette punktet omhandler helseplager som kommer mens du er på jobb eller like etter at du har vært på jobb. Selv om du har svart på lignende spørsmål tidligere i dette spørreskjemaet, bør vi deg svare på disse i tillegg.

**34. Har du i forbindelse med arbeidet du utfører hatt noen av følgende symptomer/plager siste 12 måneder? (Hvis du ikke har hatt noen symptomer setter du ingen kryss. Flere kryss er mulig)**

Ja, ofte (hver uke)      Ja, iblant

- |  |                          |                          |
|--|--------------------------|--------------------------|
| Tørrhoste  | <input type="checkbox"/> | <input type="checkbox"/> |
| Hoste med slim   | <input type="checkbox"/> | <input type="checkbox"/> |
| Piping i brystet   | <input type="checkbox"/> | <input type="checkbox"/> |
| Trykk over brystet   | <input type="checkbox"/> | <input type="checkbox"/> |
| Brystsmerter   | <input type="checkbox"/> | <input type="checkbox"/> |
| Åndenød, tett i brystet  | <input type="checkbox"/> | <input type="checkbox"/> |
| Hyppig nysing  | <input type="checkbox"/> | <input type="checkbox"/> |
| Irritert, tett eller rennende nese   | <input type="checkbox"/> | <input type="checkbox"/> |
| Heshet, sår hals eller irritasjon i halsen   | <input type="checkbox"/> | <input type="checkbox"/> |
| Tung i hodet/hodepine  | <input type="checkbox"/> | <input type="checkbox"/> |
| Kløe, svie, irritasjon i øynene  | <input type="checkbox"/> | <input type="checkbox"/> |
| Unormal tretthet   | <input type="checkbox"/> | <input type="checkbox"/> |
| Frysninger/muskelsmerter/feber uten at du har hatt influensa eller annen infeksjon | <input type="checkbox"/> | <input type="checkbox"/> |

**35. Dersom du har opplevd noen av plagene som er listet opp under spørsmål 34, under/etter hvilket arbeid eller hvilken arbeidsprosess oppstod plagene? (Flere kryss er mulig)**

- |  |                          |
|--|--------------------------|
| Bløgging                               | <input type="checkbox"/> |
| Sløyemaskin                            | <input type="checkbox"/> |
| Etterrensing                           | <input type="checkbox"/> |
| Skjæring/kutting av filet med maskiner | <input type="checkbox"/> |
| Filetkutting for hånd                  | <input type="checkbox"/> |
| Røyking av fisk                        | <input type="checkbox"/> |
| Vektsortering/kvalitetkontroll av fisk | <input type="checkbox"/> |

**Spørsmål 35 forts.**

- |   |                          |
|---|--------------------------|
| Pakkning av fisk                                    | <input type="checkbox"/> |
| Kjølerom/kjølelager                                 | <input type="checkbox"/> |
| Fryselager  | <input type="checkbox"/> |
| Håndtering av fiskeavfall                           | <input type="checkbox"/> |
| Produksjon av fiskemat/videreforedling av produkter | <input type="checkbox"/> |
| Teknisk vedlikehold av produksjonsmaskiner          | <input type="checkbox"/> |
| Laboratorium  | <input type="checkbox"/> |
| Kontor/administrasjon                               | <input type="checkbox"/> |
| Annet   | <input type="checkbox"/> |
| Hva _____   | <input type="checkbox"/> |

**36. Dersom du har opplevd noen av plagene som er listet opp under spørsmål 34, hva tror du selv kan være årsak til plagene? (Flere kryss er mulig)**

- |  |                          |
|--|--------------------------|
| Sprut/tåke fra maskiner og/eller fra dyser | <input type="checkbox"/> |
| Sprut/tåke i forbindelse med bløgging      | <input type="checkbox"/> |
| Kontakt med laks                           | <input type="checkbox"/> |
| Kontakt med slimet på utsiden av laksen    | <input type="checkbox"/> |
| Kontakt med innvoller/fiskeavfall          | <input type="checkbox"/> |
| Kalde omgivelser/kulde                     | <input type="checkbox"/> |
| Vaskemidler / desinfeksjonsmidler          | <input type="checkbox"/> |
| Spyling                                    | <input type="checkbox"/> |
| Forurensset luft                           | <input type="checkbox"/> |
| Eksos                                      | <input type="checkbox"/> |
| Annet                                      | <input type="checkbox"/> |
| Hva _____                                  | <input type="checkbox"/> |

**37. Har du noen gang skiftet arbeidsoppgaver i bedriften på grunn av luftveisplager?** Ja     Nei

Hvis ja, hvilke arbeidsoppgaver måtte du skifte fra?

**38. Bruker du hanske under arbeid?**

Nesten alltid	<input type="checkbox"/>	Av og til	<input type="checkbox"/>	Aldri	<input type="checkbox"/>
---------------	--------------------------	-----------	--------------------------	-------	--------------------------

**39. Har du i forbindelse med arbeidet du utfører hatt noen av følgende symptomer/plager siste 12 måneder? (flere kryss er mulig)**

- |                                 |                          |                     |                          |
|---------------------------------|--------------------------|---------------------|--------------------------|
| Kløe, svie, irritasjon i øynene | <input type="checkbox"/> | Tørr hud            | <input type="checkbox"/> |
| Hudkløe                         | <input type="checkbox"/> | Sprukken hud        | <input type="checkbox"/> |
| Utslett                         | <input type="checkbox"/> | Sår som gror dårlig | <input type="checkbox"/> |

**40. Hvis du har hudplager, angi hvor på kroppen du har disse plagene: (flere kryss er mulig)**

- |              |                          |              |                          |
|--------------|--------------------------|--------------|--------------------------|
| Hender       | <input type="checkbox"/> | Underarm     | <input type="checkbox"/> |
| Ansikl       | <input type="checkbox"/> | Hele kroppen | <input type="checkbox"/> |
| Andre steder | <input type="checkbox"/> |              |                          |



## GENERELT OM HELSETILSTANDEN

**15. Har du eller har du hatt en eller flere av følgende plager/sykdommer etter du fylte 15 år?**

	Hvis ja, har en lege bekreflet det?		
	Ja	Ja	Nei
Astma	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Kronisk bronkitt/emfysem/KOLS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tuberkulose	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Angina pectoris (hjertekrampe)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hjerteflimmer (atrieflimmer)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hjerteinfarkt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Høyt blodtrykk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Andre hjertesykdommer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hudeksem	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Allergi	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Reumatisk sykdom	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**16. Har du som barn hatt en eller begge av følgende plager/sykdommer? (Flere kryss er mulig)**

Astma (barneastma)?   
Eksem (atopisk eksem)?

**17. Hvis du er allergisk, hva er du allergisk mot? (Flere kryss er mulig)**

Laks	<input type="checkbox"/>	Pollen, gress	<input type="checkbox"/>
Sild	<input type="checkbox"/>	Støv	<input type="checkbox"/>
Torsk	<input type="checkbox"/>	Mat	<input type="checkbox"/>
Reker/skalldyr	<input type="checkbox"/>	Dyr	<input type="checkbox"/>
Annen fisk	<input type="checkbox"/>	Annet (hva)	<input type="checkbox"/>

**18. Har du i løpet av de siste 12 måneder hatt piping i brystet, som ikke var forkjølelse eller influensa?**

Ja  Nei

**19. Hvis ja på spørsmål 18, var du tungpustet også?**

Ja  Nei

**20. Hoster eller harker (kremter) du vanligvis om morgenen?**

Ja  Nei

**21. Hvis ja på spørsmål 20, har du vanligvis oppspyt?**

Ja  Nei

**22. Hoster du nærmest daglig til sammen 3 måneder eller lenger i løpet av et år?**

Ja  Nei

**23. Har du i løpet av de siste 12 månedene hatt rennende eller tett nese som ikke har vært forkjølelse eller influensa?**

Ja  Nei

Hvis nei, gå til spørsmål 30

**24. Hvis ja på spørsmål 23, har du samtidig hatt**

Kløende, rennende øyne	<input type="checkbox"/>	Ja	<input type="checkbox"/>	Nei
Søvnproblemer	<input type="checkbox"/>		<input type="checkbox"/>	

**25. Hvis ja på spørsmål 23, tror du at det kan være spesielle forhold som f. eks lukt, irriterende stoffer, temperatur o.l., som forårsaker neseplagene?**

Ja  Nei +

Hvis ja, hvilke forhold du tror det er \_\_\_\_\_

**26. Hvis ja på spørsmål 23, i hvilken av de siste 12 månedene har du hatt plagene?**

Januar	<input type="checkbox"/>	Mai	<input type="checkbox"/>	September	<input type="checkbox"/>
Februar	<input type="checkbox"/>	Juni	<input type="checkbox"/>	Oktober	<input type="checkbox"/>
Mars	<input type="checkbox"/>	Juli	<input type="checkbox"/>	November	<input type="checkbox"/>
April	<input type="checkbox"/>	August	<input type="checkbox"/>	Desember	<input type="checkbox"/>

**27. Hvis ja på spørsmål 23, når oppstår neseplagene?**

	Ja	Nei
Hvert år, og alltid på samme årstid	<input type="checkbox"/>	<input type="checkbox"/>
I forbindelse med arbeidet ditt	<input type="checkbox"/>	<input type="checkbox"/>
Hvis ja, forsvinner plagene i helger og ferier?	<input type="checkbox"/>	<input type="checkbox"/>
I forbindelse med bruk av Dispril eller andre smertestillende medisiner	<input type="checkbox"/>	<input type="checkbox"/>
Hvis ja, hvilke medisiner		

**28. Hvis du har hatt neseplager siste 12 måneder, hvor ofte har du hatt disse plagene?**

Mindre enn 4 dager pr uke eller til sammen mindre enn 4 uker siste år

Mer enn 4 dager pr uke og til sammen mer enn 4 uker siste år

**29. Hvis du har hatt neseplager siste 12 måneder, har de hemmet deg i dine daglige gjøremål som skole, arbeid, fritidsaktiviteter og/eller sport?**

Nei, ikke i det hele tatt   
Litt   
Mye

**30. Har du noen gang hatt**

	Ja	Nei
Høysnue	<input type="checkbox"/>	<input type="checkbox"/>
Astma	<input type="checkbox"/>	<input type="checkbox"/>
Hudallergi	<input type="checkbox"/>	<input type="checkbox"/>

**31. Har noen i din familie noen gang hatt**

	Ja	Nei
Astma	<input type="checkbox"/>	<input type="checkbox"/>
Hudallergi	<input type="checkbox"/>	<input type="checkbox"/>
Neseallergi	<input type="checkbox"/>	<input type="checkbox"/>

## RØYKING

### 41. Røykevaner (ett kryss)

Røyker daglig  Har røkt tidligere   
Røyker av og til  Nei, har aldri røkt



Hvis nei, har aldri røkt; gå til spørsmål 46.

### 42. Hvis du har røkt tidligere, hvor mange år er det siden du sluttet?

Antall år

### 43. Hvor mange sigarettter røyker eller rakte du vanligvis daglig?

Antall sigarettter

### 44. Hvor gammel var du da du begynte å røyke daglig?

År

### 45. I hvor mange år til sammen har du røkt?

Antall år



## TERMISK MILJØ

### 46. Fryser du når du er på arbeid?



Ja, ofte  Ja, iblant  Nei, sjeldent/aldri

### 47. Hvor oppholder du deg mesteparten av arbeidstiden din? (Ett kryss)

Oppvarmet lokale

Ikke oppvarmet lokale

Kjølelager/utendørs

### 48. Besvares hvis du jobber mesteparten av tiden på kjølelager eller utendørs: Har du noen gang opplevd noen av disse symptomene mens du oppholder deg i kjølelager/utendørs?



Ja

Nei

## **Appendix D**

Questionnaire for crab processing workers (English and Norwegian)



# **WORK ENVIRONMENT AND HEALTH IN THE CRAB INDUSTRY**

## Reference number

## PERSONAL

1. Sex:      Male       Female

2. Year of birth (e.g. 1963)

3. Are you a Norwegian or Nordic citizen with permanent residence in Norway? Yes  No

4. Have you lived continuously in Norway for the last five years? Yes  No

5. How many years of education do you have in total (included elementary school, junior high, high school, and further studies)  years

6. Do you eat crab? Yes  No

7. If yes, how often do you eat crab during the crab season? \_\_\_\_\_ times pr week  
\_\_\_\_\_ times pr month

8. Do you work in the crab industry? Yes  No   
*If no, move on to question number 16*

9. Have you worked in the sea food industry earlier? Yes  No

10. If yes, what kind of sea food industry?  
Crab   
White fish   
Herring   
Salmon   
Shrimp   
Other

## GENERALLY ON WORK CONDITIONS

11. In total, how many years have you worked in the crab Industry?  years

12. In which part of the plant do you work?

Yes, more than  
half the time

Yes, less than  
half the time

Treatment of raw crab

Treatment of boiled crab

Administration/office

Other

What? \_\_\_\_\_

13. What are your work assignments?

Yes, more than  
half the time

Yes, less than  
half the time

Washing/cleaning raw crab

Slaughter/cutting/removing claws of raw crab

Boiling crab

Treatment/further processing of boiled crab

Packing

Glazing/icing

Freezer/cold store

Handling of waste

Technical maintenance of production machines

Laboratory

Office/administration

Other

What? \_\_\_\_\_

14. Do you work with water jets for cleaning (e. g. floors, machines) at your work place?

Yes, often

Yes, sometimes

No, rarely

If yes, often, how often? \_\_\_\_\_ times pr day

15. Do you use masks when you work?

Always

Sometimes

Rarely/never

**16.What level of activity have you usually had at work in the last 12 months? (one mark possible)**

- Moderate physical activity, mostly sedentary/standing work
- (e.g. work in the office or similar non strenuous activities)
- Work that requires a lot of walking
- Work that requires a lot of walking and lifting
- Physically strenuous work

**GENERALLY ON YOUR HEALTH**

**17. Do you have, or have you ever had, any of the following medical conditions since the age of 15 years old? (several marks possible)**

	If yes, has it been affirmed by a doctor?		
Yes	Yes	No	
Asthma	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chronic bronchitis/emphysema/COPD	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tuberculosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Angina pectoris (heart cramp)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cardiac fibrillation (atrial fibrillation)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Myocardial infarction	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
High blood pressure/hypertension	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other heart conditions	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Skin eczema	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Allergies	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Rheumatic illness	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**18. If you have asthma, can you estimate how many asthma attacks you've had in the last 12 months:**

Asthma attacks

**19. Did you as a child have any of the following medical conditions? (several marks possible)**

- Asthma (children's asthma)?
- Eczema (atopic eczema)?
- Allergies

**20. If you are allergic, what are you allergic to? (several marks possible)**

Crab

Pollen, grass

Shrimp/other shell fish

Fungi/dust/mites

Fish

Food

Animals

Other  What? \_\_\_\_\_

Yes  No

**21. Did you experience wheezing in the last 12 months?**

**22. If you answered yes on question 21,  
did you have trouble breathing too?**

**23. Do you usually cough (hem) in the morning?**

**24. If you answered yes on question 23,  
do you usually have expectoration?**

**25. Do you cough daily/almost daily on average  
3 months or more during a year?**

**26. Did you in the course of the last 12 months experience  
a runny nose or nasal congestion that has not been  
in association with a cold or the flu?** Yes  No

If you answered no to question 26, skip ahead to question 33

**27. If you answered yes to question 26, have you at the same time had any of the following:**

	Yes <input type="checkbox"/>	No <input type="checkbox"/>
Itchy, runny eyes	<input type="checkbox"/>	<input type="checkbox"/>
Sleeping problems	<input type="checkbox"/>	<input type="checkbox"/>

**28. If you answered yes to question 26, do you think that it might be any particular reasons causing these  
nasal problems (e.g. smells, irritating particles, temperature)**

Yes <input type="checkbox"/>	No <input type="checkbox"/>
------------------------------	-----------------------------

If yes, what reasons do you think it is? \_\_\_\_\_

**29. If you answered yes to question 26, in which one of the last 12 months have you had these symptoms?**

January

May

September

February

June

October

March

July

November

April

August

December

**30. If you answered yes to question 26, when do the nasal problems appear?**

	Yes	No
Every year, and always at the same time of year	<input type="checkbox"/>	<input type="checkbox"/>
In association with your work	<input type="checkbox"/>	<input type="checkbox"/>

If the problems appear in association  
with your work, do the problems disappear during  
the weekend and holidays?

Yes	No
<input type="checkbox"/>	<input type="checkbox"/>

In association with use of dispril or any  
other pain killing tablets

Yes	No
<input type="checkbox"/>	<input type="checkbox"/>

*If yes, which tablets \_\_\_\_\_*

**31. If you have experienced nasal problems in the last 12 months, how often did you experience these problems?**

Less than 4 days pr week or less than 4 weeks during this last year in total	<input type="checkbox"/>
More than 4 days pr week and more than 4 weeks this last year in total	<input type="checkbox"/>

**32. If you have experienced nasal problems in the last 12 months, has it interferred with your daily activities such as school, work, after hours activities and/or sports?**

No, not at all	<input type="checkbox"/>
A little	<input type="checkbox"/>
Some	<input type="checkbox"/>
A lot	<input type="checkbox"/>

**33. Has anyone in your family ever had any of the following:**

	Yes	No
Asthma	<input type="checkbox"/>	<input type="checkbox"/>
Skin allergies	<input type="checkbox"/>	<input type="checkbox"/>
Nasal allergies	<input type="checkbox"/>	<input type="checkbox"/>

**34. How would you assess your general health? (one mark)**

Very good	<input type="checkbox"/>
Good	<input type="checkbox"/>
Neither good nor bad	<input type="checkbox"/>
Bad	<input type="checkbox"/>
Very bad	<input type="checkbox"/>

**35. How would you describe your health, compared to others your age? (one mark)**

Much better	<input type="checkbox"/>
A little better	<input type="checkbox"/>
About the same	<input type="checkbox"/>
A little worse	<input type="checkbox"/>
Much worse	<input type="checkbox"/>

## HEALTH PROBLEMS IN ASSOCIATION WITH YOUR WORK

The questions below this point concerns health problems that arrive while you are at work or just after you've been at work. Even if you've already answered similar questions earlier in this questionnaire, we ask you to answer these too.

- 36. Have you, in association with your work, had any of the following symptoms/ailments in the last 12 months? (If you haven't had any symptoms, you don't mark any boxes. Several marks are possible)**

Yes, often (every week)      Yes, sometimes

Dry cough	<input type="checkbox"/>	<input type="checkbox"/>
Cough with slime	<input type="checkbox"/>	<input type="checkbox"/>
Wheezing	<input type="checkbox"/>	<input type="checkbox"/>
Chest tightness	<input type="checkbox"/>	<input type="checkbox"/>
Chest pains	<input type="checkbox"/>	<input type="checkbox"/>
Problems breathing, dyspnea	<input type="checkbox"/>	<input type="checkbox"/>
Frequent sneezing	<input type="checkbox"/>	<input type="checkbox"/>
Nasal congestion, irritated or runny nose	<input type="checkbox"/>	<input type="checkbox"/>
Hoarseness, sore throat or irritations in the throat	<input type="checkbox"/>	<input type="checkbox"/>
Headache or "heavy head"	<input type="checkbox"/>	<input type="checkbox"/>
Itching, burning or irritations in the eyes	<input type="checkbox"/>	<input type="checkbox"/>
Abnormal tiredness	<input type="checkbox"/>	<input type="checkbox"/>
Chills/muscle ache/ fever without having influenza or any other infections	<input type="checkbox"/>	<input type="checkbox"/>

- 37. If you have experienced any of the symptoms listed in question 36, do these symptoms disappear during weekends and holidays?**

Yes       No

- 38. If you have experienced any of the symptoms listed in question 36, during/after which kind of work did the symptoms appear? (Several marks possible)**

Washing/cleaning raw crab	<input type="checkbox"/>
Slaughtering/cutting/removing claws, raw crab	<input type="checkbox"/>
Boiling crab	<input type="checkbox"/>
Treatment of boiled crab	<input type="checkbox"/>
Packing	<input type="checkbox"/>
Glazing/icing	<input type="checkbox"/>
Freezer, cold storage	<input type="checkbox"/>
Handling of waste	<input type="checkbox"/>
Technical maintenance of production machines	<input type="checkbox"/>
Laboratory	<input type="checkbox"/>
Office/administration	<input type="checkbox"/>
Other	<input type="checkbox"/> What? _____

**39. If you have experienced any of the symptoms listed in question 36, what do you think the cause of these symptoms might be? (Several marks possible)**

- Splash from machines and/or from nozzles
- Contact with crab
- Contact with intestines/waste
- Cold surroundings/cold
- Detergents/disinfectants
- Use of water jets
- Polluted air
- Exhaust
- Other  What? \_\_\_\_\_

**40. Have you ever changed work assignments in the work place because of airway problems?**

Yes  No

*If yes, which work assignments did you have to change from? \_\_\_\_\_*

**41. Do you use gloves when performing your work?**

Almost always  Sometimes  Never

**42. Have you in connection with the work you perform had any of the following symptoms/ailments in the last 12 months? (several marks is possible)**

- Itching, burning or irritation in the eyes   
Cracked skin   
Rash  Dry skin   
Itching skin   
Wounds that heal poorly

**43. If you've had skin symptoms, where on the body have these conditions been located? (several marks possible)**

- Hands   
Face   
Other places  Forearm   
Whole body

## SMOKING

**44. Smoking habits (only one mark)**

Smoke daily  Smoke sometimes, but not daily  Have smoked earlier  No, I have never smoked

*If you answered No, I have never smoked, move on to question 48.*

**45. If you have smoked earlier, how many years has it been since you quit?**

Number of years

**46. How many cigarettes do you/did you smoke on average each day?**

Number of cigarettes

**47. How old were you when you started smoking every day?**

Age

**48. How many years have you smoked, combined?**

Number of years

## THERMAL ENVIRONMENT

**49. Do you feel cold at work?**

Yes, often  Yes, sometimes  No, rarely/never

**50. Where do you spend most of your working hours? (one mark)**

Heated areas  Unheated areas  Cooling areas/out doors

**51. Answer this question if you work most of your time in cooling storages or out doors:**

**Have you ever experienced any of these symptoms while you are in a cool storage or out doors?**

	Yes	No
Respiratory problems	<input type="checkbox"/>	<input type="checkbox"/>
Repeated coughing	<input type="checkbox"/>	<input type="checkbox"/>
Wheezing	<input type="checkbox"/>	<input type="checkbox"/>
Mucus from the lungs	<input type="checkbox"/>	<input type="checkbox"/>
Chest pains	<input type="checkbox"/>	<input type="checkbox"/>
Disturbances in the heart rhythm	<input type="checkbox"/>	<input type="checkbox"/>
Decreased blood circulation in your hands/feet	<input type="checkbox"/>	<input type="checkbox"/>

# ARBEIDSMILJØ OG HELSE I KRABBEINDUSTRIEN

Referanse nr

## PERSONALIA

1. **Kjønn:** Mann  Kvinne
2. **Fødselsår (f.eks. 1963)**
3. **Er du norsk eller nordisk statsborger med fast bosted i Norge?** Ja  Nei
4. **Har du bodd sammenhengende i Norge de siste fem årene?** Ja  Nei
5. **Hvor mange års utdanning har du totalt (inkludert barneskole, ungdomsskole, videregående skole, senere skolegang/studier)**  år
6. **Spiser du krabbe?** Ja  Nei
7. **Hvis ja, hvor ofte spiser du krabbe i sesongen?** \_\_\_\_\_ ganger pr uke  
\_\_\_\_\_ ganger pr måned
8. **Jobber du i krabbeindustrien?** Ja  Nei   
*Hvis nei, gå til spørsmål 16*
9. **Har du jobbet i sjømatindustrien tidligere?** Ja  Nei
10. **Hvis ja, angi hvilken type sjømat**  
Krabbe   
Hvitfisk   
Sild   
Laks   
Reke   
Annet

## GENERELT OM ARBEIDSFORHOLD

11. I hvor mange år har du totalt jobbet i krabbeindustrien?  år

12. I hvilken del av bedriften jobber du ?

Ja, mer enn  
halvparten av tida      Ja, mindre enn  
halvparten av tida

- |                           |                          |                          |
|---------------------------|--------------------------|--------------------------|
| Behandling av rå krabbe   | <input type="checkbox"/> | <input type="checkbox"/> |
| Behandling av kokt krabbe | <input type="checkbox"/> | <input type="checkbox"/> |
| Administrasjon/kontor     | <input type="checkbox"/> | <input type="checkbox"/> |
| Annet                     | <input type="checkbox"/> | Hva: _____               |

13. Hvilke arbeidsoppgaver har du?

Ja, mer enn  
halvparten av tida      Ja, mindre enn  
halvparten av tida

- |  |                          |                          |
|--|--------------------------|--------------------------|
| Vasking/ rensing av rå krabbe                | <input type="checkbox"/> | <input type="checkbox"/> |
| Slakting/kutting/fjerning av klør, rå krabbe | <input type="checkbox"/> | <input type="checkbox"/> |
| Koking av krabbe                             | <input type="checkbox"/> | <input type="checkbox"/> |
| Behandling/videreforedling av kokt krabbe    | <input type="checkbox"/> | <input type="checkbox"/> |
| Pakking                                      | <input type="checkbox"/> | <input type="checkbox"/> |
| Glassering                                   | <input type="checkbox"/> | <input type="checkbox"/> |
| Fryselager                                   | <input type="checkbox"/> | <input type="checkbox"/> |
| Håndtering av avfall                         | <input type="checkbox"/> | <input type="checkbox"/> |
| Teknisk vedlikehold av produksjonsmaskiner   | <input type="checkbox"/> | <input type="checkbox"/> |
| Laboratorium                                 | <input type="checkbox"/> | <input type="checkbox"/> |
| Kontor/administrasjon                        | <input type="checkbox"/> | <input type="checkbox"/> |
| Annet  | <input type="checkbox"/> | Hva: _____               |

14. Arbeider du med spyling (f. eks gulv, maskiner) på din arbeidsplass?

Ja, ofte       Ja, iblant       Nei, sjeldent

Hvis ja, ofte, hvor ofte? \_\_\_\_\_ ganger pr dag

15. Bruker du maske/munnnbind når du jobber? Alltid  Av og til  Sjeldent/aldri

16. Hva slags aktivitet har du vanligvis hatt i arbeidet ditt siste 12 måneder? (ett kryss)

Lett fysisk aktivitet, for det meste stillesittende/stående arbeid

(f.eks kontorarbeid eller tilsvarende lett aktivitet)

- Arbeid som krever at du går mye
- Arbeid som krever at du går og løfter mye
- Tungt fysisk arbeid

## GENERELT OM HELSETILSTANDEN

**17. Har du eller har du hatt en eller flere av følgende plager/sykdommer etter du fylte 15 år?**  
*(flere kryss er mulig)*

		Hvis ja, har en lege bekreftet det?	
	Ja	Ja	Nei
Astma	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Kronisk bronkitt/emfysem/KOLS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tuberkulose	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Angina pectoris (hjertekrampe)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hjerteflimmer (atrieflimmer)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hjerteinfarkt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Høyt blodtrykk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Andre hjertesykdommer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hudeksem	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Allergi	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Reumatisk sykdom	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**18. Hvis du har astma, kan du anslå hvor mange astma-anfall har du hatt siste 12 måneder:**

**19. Har du som barn hatt en eller flere av følgende plager/sykdommer? (flere kryss er mulig)**

- Astma (barneastma)?
- Eksem (atopisk eksem)?
- Allergi

**20. Hvis du er allergisk, hva er du allergisk mot? (flere kryss er mulig)**

- |  |  |
|--|--|
| Krabbe <input type="checkbox"/>              | Pollen, gress <input type="checkbox"/>   |
| Reke/andre skalldyr <input type="checkbox"/> | Sopp/støv/midd <input type="checkbox"/>  |
| Fisk <input type="checkbox"/>                | Mat <input type="checkbox"/>             |
| Dyr <input type="checkbox"/>                 | Annet <input type="checkbox"/> Hva _____ |

	Ja	Nei
<b>21. Har du i løpet av de siste 12 måneder hatt piping i brystet?</b>	<input type="checkbox"/>	<input type="checkbox"/>
<b>22. Hvis ja på spørsmål 21, var du tungpustet også?</b>	<input type="checkbox"/>	<input type="checkbox"/>
<b>23. Hoster eller harker (kremter) du vanligvis om morgenen?</b>	<input type="checkbox"/>	<input type="checkbox"/>
<b>24. Hvis ja på spørsmål 23, har du vanligvis oppspyt?</b>	<input type="checkbox"/>	<input type="checkbox"/>
<b>25. Hoster du daglig/nærmest daglig til sammen 3 måneder eller lenger i løpet av et år?</b>	<input type="checkbox"/>	<input type="checkbox"/>
 <b>26. Har du i løpet av de siste 12 månedene hatt rennende eller tett nese som ikke har vært forkjølelse eller influensa?</b>	Ja <input type="checkbox"/>	Nei <input type="checkbox"/>

Hvis nei, gå til spørsmål 33

<b>27. Hvis ja på spørsmål 26, har du samtidig hatt kløende, rennende øyne</b>	Ja <input type="checkbox"/> <input type="checkbox"/>	Nei <input type="checkbox"/> <input type="checkbox"/>
<b>28. Hvis ja på spørsmål 26, tror du at det kan være spesielle faktorer (lukt, irriterende stoffer, temperatur o.l.) som forårsaker neseplagene?</b>	Ja <input type="checkbox"/>	Nei <input type="checkbox"/>

Hvis ja, hvilke faktorer du tror det er? \_\_\_\_\_

**29. Hvis ja på spørsmål 26, i hvilken av de siste 12 månedene har du hatt disse symptomene?**

Januar <input type="checkbox"/>	Mai <input type="checkbox"/>	September <input type="checkbox"/>
Februar <input type="checkbox"/>	Juni <input type="checkbox"/>	Oktober <input type="checkbox"/>
Mars <input type="checkbox"/>	Juli <input type="checkbox"/>	November <input type="checkbox"/>
April <input type="checkbox"/>	August <input type="checkbox"/>	Desember <input type="checkbox"/>

<b>30. Hvis ja på spørsmål 26, når oppstår neseplagene?</b>	Ja	Nei
Hvert år, og alltid på samme årstid	<input type="checkbox"/>	<input type="checkbox"/>
I forbindelse med arbeidet ditt	<input type="checkbox"/>	<input type="checkbox"/>
Hvis plagene oppstår i forbindelse med arbeidet, forsvinner plagene i helger og ferier?	<input type="checkbox"/>	<input type="checkbox"/>
I forbindelse med bruk av dispril eller andre smertestillende medisiner	<input type="checkbox"/>	<input type="checkbox"/>

Hvis ja, hvilke medisiner \_\_\_\_\_

**31. Hvis du har hatt neseplager siste 12 måneder, hvor ofte har du hatt disse plagene?**

Mindre enn 4 dager pr uke eller til sammen mindre enn 4 uker siste år   
Mer enn 4 dager pr uke og til sammen mer enn 4 uker siste år

**32. Hvis du har hatt neseplager siste 12 måneder, har de hemmet deg i dine daglige gjøremål som skole, arbeid, fritidsaktiviteter og/eller sport?**

Nei, ikke i det hele tatt   
Litt   
Noe, endel   
Mye

**33. Har noen i din familie noen gang hatt**

	Ja	Nei
Astma	<input type="checkbox"/>	<input type="checkbox"/>
Hudallergi	<input type="checkbox"/>	<input type="checkbox"/>
Neseallergi	<input type="checkbox"/>	<input type="checkbox"/>

**34. Hvordan vurderer du din egen helse sånn i alminnelighet? (ett kryss)**

Meget god   
God   
Verken god eller dårlig   
Dårlig   
Meget dårlig

**35. Hvordan synes du at din helse er sammenlignet med andre på samme alder? (ett kryss)**

Mye bedre   
Litt bedre   
Omtrent lik   
Litt dårligere   
Mye dårligere

## HELSEPLAGER I FORBINDELSE MED ARBEIDET

Spørsmålene under dette punktet omhandler helseplager som kommer mens du er på jobb eller like etter du har vært på jobb. Selv om du har svart på lignende spørsmål tidligere i dette spørreskjemaet, ber vi deg svare på disse i tillegg.

- 36. Har du i forbindelse med arbeidet du utfører hatt noen av følgende symptomer/plager siste 12 måneder?** (Hvis du ikke har hatt noen symptomer setter du ingen kryss. Flere kryss er mulig)

	Ja, ofte (hver uke)	Ja, iblant
Tørrhoste	<input type="checkbox"/>	<input type="checkbox"/>
Hoste med slim	<input type="checkbox"/>	<input type="checkbox"/>
Piping i brystet	<input type="checkbox"/>	<input type="checkbox"/>
Trykk over brystet	<input type="checkbox"/>	<input type="checkbox"/>
Brystsmerter	<input type="checkbox"/>	<input type="checkbox"/>
Åndenød, tett i brystet	<input type="checkbox"/>	<input type="checkbox"/>
Hyppig nysing	<input type="checkbox"/>	<input type="checkbox"/>
Irritert, tett eller rennende nese	<input type="checkbox"/>	<input type="checkbox"/>
Heshet, sår hals eller irritasjon i halsen	<input type="checkbox"/>	<input type="checkbox"/>
Tung i hodet/hodepine	<input type="checkbox"/>	<input type="checkbox"/>
Kløe, svie, irritasjon i øynene	<input type="checkbox"/>	<input type="checkbox"/>
Unormal tretthet	<input type="checkbox"/>	<input type="checkbox"/>
Frysninger/muskelsmerter/feber uten at du har hatt influensa eller annen infeksjon	<input type="checkbox"/>	<input type="checkbox"/>

- 37. Hvis du har opplevd noen av plagene som er listet opp under spørsmål 36, forsvinner plagene i løpet av helger og ferier?**

Ja  Nei

- 38. Hvis du har opplevd noen av plagene som er listet opp under spørsmål 36, under/etter hvilket arbeid eller hvilken arbeidsprosess oppstod plagene? (Flere kryss er mulig)**

Vasking/ rensing av rå krabbe	<input type="checkbox"/>
Slakting/kutting/fjerning av klør, rå krabbe	<input type="checkbox"/>
Koking av krabbe	<input type="checkbox"/>
Behandling av kokt krabbe med skall	<input type="checkbox"/>
Pakking	<input type="checkbox"/>
Glassering	<input type="checkbox"/>
Fryselager	<input type="checkbox"/>
Håndtering av avfall	<input type="checkbox"/>
Teknisk vedlikehold av produksjonsmaskiner	<input type="checkbox"/>
Laboratorium	<input type="checkbox"/>
Kontor/administrasjon	<input type="checkbox"/>
Annet	<input type="checkbox"/> Hva? _____

**30. Hvis du har opplevd noen av plagene som er listet opp under spørsmål 36, hva tror du selv kan være årsak til plagene? (Flere kryss er mulig)**

- Sprut fra maskiner og/eller fra dyser
- Kontakt med krabbe
- Kontakt med innvoller/avfall
- Kalde omgivelser/kulde
- Vaskemidler / desinfeksjonsmidler
- Spyling
- Forurensset luft
- Eksos
- Annet  Hva? \_\_\_\_\_

**40. Har du noen gang skiftet arbeidsoppgaver i bedriften på grunn av luftveisplager?**

Ja  Nei

Hvis ja, hvilke arbeidsoppgaver måtte du skifte fra? \_\_\_\_\_

**41. Bruker du hansker under arbeid?**

Nesten alltid  Av og til  Aldri

**42. Har du i forbindelse med arbeidet du utfører hatt noen av følgende symptomer/plager siste 12 måneder? (flere kryss er mulig)**

- Kløe, svie, irritasjon i øynene   
Sprukken hud   
Utslett  Tørr hud   
 Hudkløe   
 Sår som gror dårlig

**43. Hvis du har hudplager, angi hvor på kroppen du har disse plagene: (flere kryss er mulig)**

- Hender   
Ansikt   
Andre steder  Underarm   
 Hele kroppen

**RØYKING**

**44. Røykevaner (sett bare ett kryss)**

Røyker daglig  Røyker av og til, men ikke daglig  Har røkt tidligere  Nei, har aldri røkt

Hvis nei, har aldri røkt; gå til spørsmål 48.

**45. Hvis du har røkt tidligere, hvor mange år er det siden du sluttet?**

Antall år

**46. Hvor mange sigareetter røyker eller røkte du vanligvis daglig?**

Antall sigareetter

**47. Hvor gammel var du da du begynte å røyke daglig?**

Antall år

**48. I hvor mange år til sammen har du røkt?**

Antall år

**TERMISK MILJØ**

**49. Fryser du når du er på arbeid?**

Ja, ofte  Ja, iblant  Nei, sjeldent/aldri

**50. Hvor oppholder du deg mesteparten av arbeidstiden din? (ett kryss)**

Oppvarmet lokale  Ikke oppvarmet lokale  Kjølelager/utendørs

**51. Besvares hvis du jobber mesteparten av tiden på kjølelager eller utendørs:**

**Har du noen gang opplevd noen av disse symptomer mens du oppholder deg i kjølelager/utendørs?**

	Ja	Nei
Pusteproblemer	<input type="checkbox"/>	<input type="checkbox"/>
Langvarig hoste	<input type="checkbox"/>	<input type="checkbox"/>
Pipende pust	<input type="checkbox"/>	<input type="checkbox"/>
Slim fra lungene	<input type="checkbox"/>	<input type="checkbox"/>
Brystsmerter	<input type="checkbox"/>	<input type="checkbox"/>
Forstyrrelse i hjerterytmen	<input type="checkbox"/>	<input type="checkbox"/>
Nedsatt blodsirkulasjon i hender/fötter	<input type="checkbox"/>	<input type="checkbox"/>



