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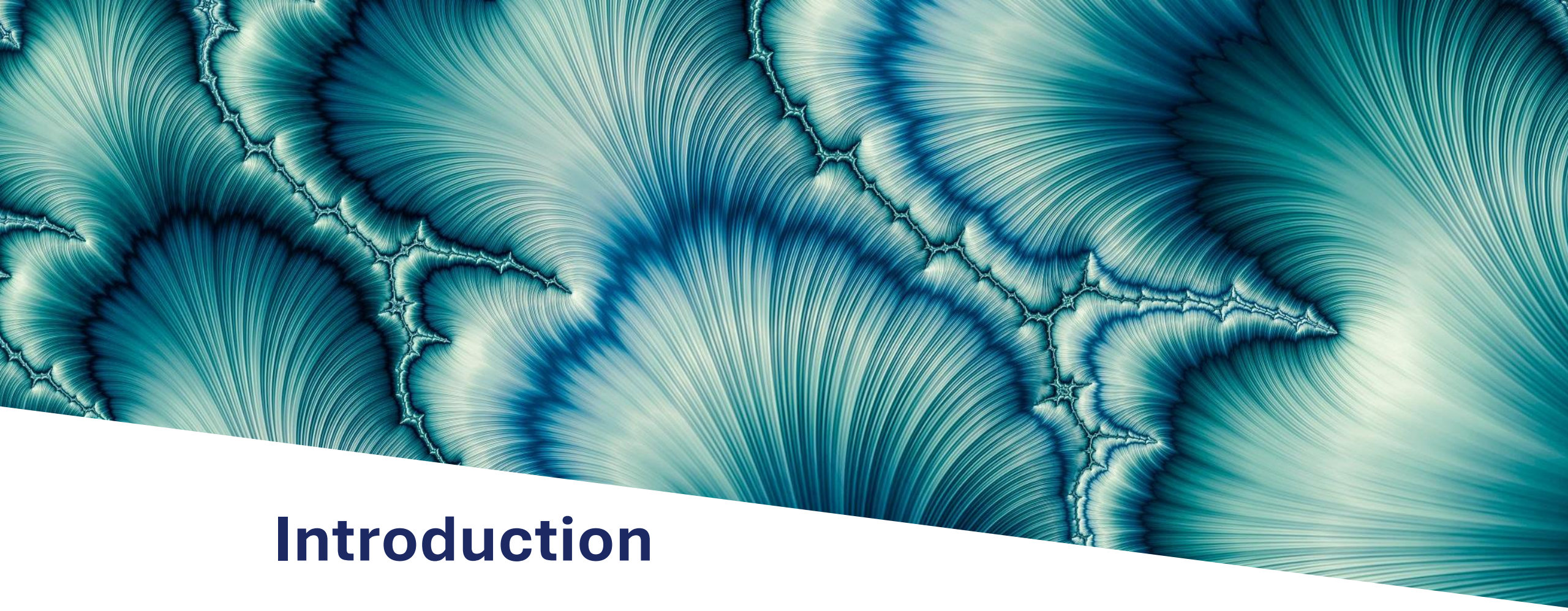
Respiratory symptoms and exposure to allergenic proteins in shrimp processing plants

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Outline

- Introduction
- Objectives
- Material and Methods
- Results
- Summary and conclusion



Introduction

The seafood industry and health problems for employees

- The seafood industry is a large part of the Norwegian food industry.
- High frequency of respiratory problems such as allergies and asthma in workers in the seafood industry.
- There is a large variation between marine species in their ability to induce respiratory symptoms.
- Workers in the shellfish industry have a higher frequency (4-36%) and more severe respiratory symptoms and are more often sensitized than those in the fishing industry (2-8%).

Jeebhay MF, Cartier A. Current Opinions in Allergy and Clinical Immunology. 2010:10:104-13

Exposure in the Shrimp Industry

- The work is characterized by a complex exposure to chemical and biological factors in addition to working in cold and wet environments.
- The bioaerosol contains allergens, microorganisms, proteases, additive preservatives, and chemicals.
- Few studies on the composition of the bioaerosols. Focus on the total protein level, the endotoxin level and the inhalable fraction that contributes to respiratory problems in employees.
- Little information about the individual protein components and their allergenic properties.

Jeebhay, 2010, Dahlman- Hoglund, 2020, Lopata, 2013, Jeebhay, 2004, Thomassen, 2017

Objectives



- To measure and characterize the composition of allergens and proteases.
- To identify work tasks and areas in production that are particularly exposed to adverse exposure.
- To identify differences in reported respiratory symptoms between exposed and unexposed workers.

Materials and Methods

- **Sampling and data collection**
- Two rounds of sampling in the two shrimp processing plants

  - Questionnaire: 65 participants
    - 34 Production workers (Exposed)
    - 10 Partly exposed office workers
    - 21 Controls /unexposed workers

  - Blood Samples: 27 (exposed), 6 (partly exposed) and 21 (controls/unexposed workers).

  - Air Samples: 31 full-shift personal samples and 12 stationary samples (11-23 h sampling).



Pandalus borealis **(Northern shrimp)**

Image: Euro Fish Magazine

Work processes in shrimp processing plant





Thawing

1



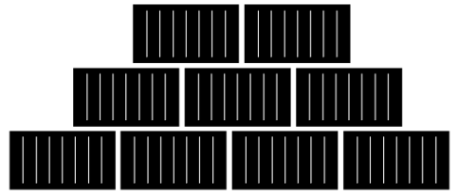
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Thawer (TH)

Truck driver (TR)

Maturation



Cooking and peeling



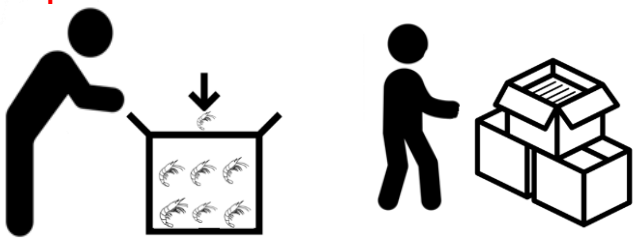
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Technician (TE)

Packing

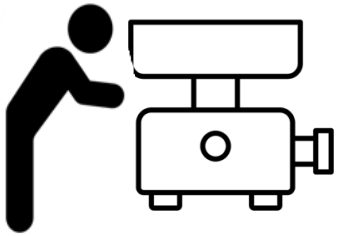
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Packers (PA)

Flour production (FL)

5



Miller

Air Sampling

- Air samples were collected using a conical Inhalable Sampler (CIS)
- 37 mm polytetrafluorethylene (PTFE) filter with a pore size of 1.0 µm.



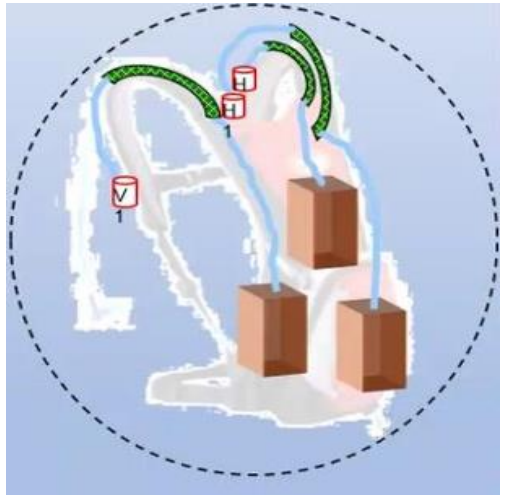


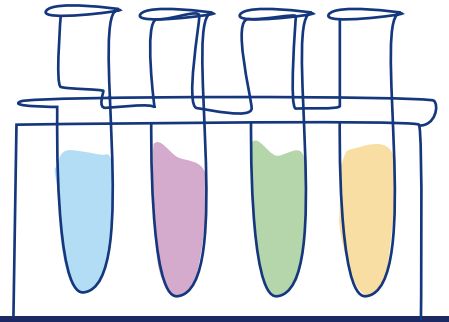




Photo AMA UNN

Sample processing and analysis

- Total protein quantification - BCA
- Protein identification - LC-MS
- Tropomyosin - ELISA
- Protease activity detection - Zymography





Results

Characteristics of Study Population

	Number (N)	Mean age yrs. ± SD	Gender, Male /Female %	Mean BMI ±SD	Current smokers %	Ex Smokers %	Mean employment yrs. ± SD
Exposed workers	34	39±14	78/22	28±7,5	12	50	8.2±11
Partly exposed workers	10	47±13	70/30	27±4,7	0	30	15±14
Control (unexposed)	21	43±8	76/24	26±3,3	9,5	56	6±5
Total (N)	**65**						

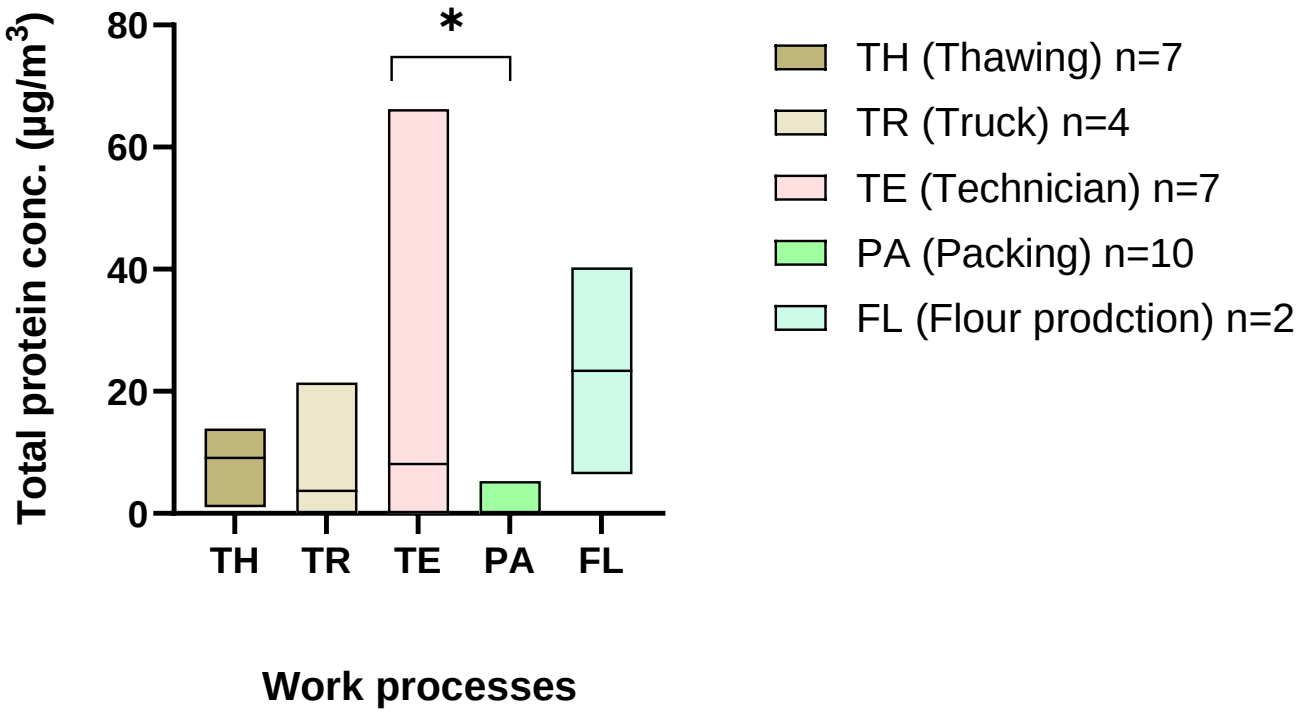
Work-related respiratory symptoms

	Exposed production workers N = 34	Partly exposed workers N = 10	Control (Unexposed) N = 21
Allergy (%)	27.3	10	42.9
Asthma diagnosis by GP (%)	24	10	4.8
Asthma attack in the past year (%)	2.9	0	0
Asthma medication use (%)	5.9	10	0
Chronic lower respiratory symptoms (%)	62*	50	29
Acute upper respiratory symptoms (%)	68 *	33	33
Acute lower respiratory symptoms (%)	35	33	14
Crab IgE ≥ 0.35 kU/L (%)	11.1	0	NA
Shrimp IgE ≥ 0.35 kU/L (%)	11.1	0	NA
Salmon IgE ≥ 0.35 kU/L (%)	3.7	0	NA

NA- Not applicable, SD- Standard deviation, GP- General practitioner, * $p < 0.05$

Chronic lower resp. symptoms: trouble breathing, shortness of breath, wheezing/ peeping sound, cough and production of phlegm in the morning, and cough daily for three months or more.
Acute upper resp. symptoms: Sneezing, Stuffy nose, Runny nose and sore throat. **Acute lower resp. symptoms:** Coughing with Phlegm or sputum, wheezing and congested chest

Characterization of Bioaerosols



Known shellfish allergens identified

- Identified a total of 95 shrimp proteins

- 15 were allergens or potential novel allergens:
  - 7 known allergens + 8 predicted novel allergens

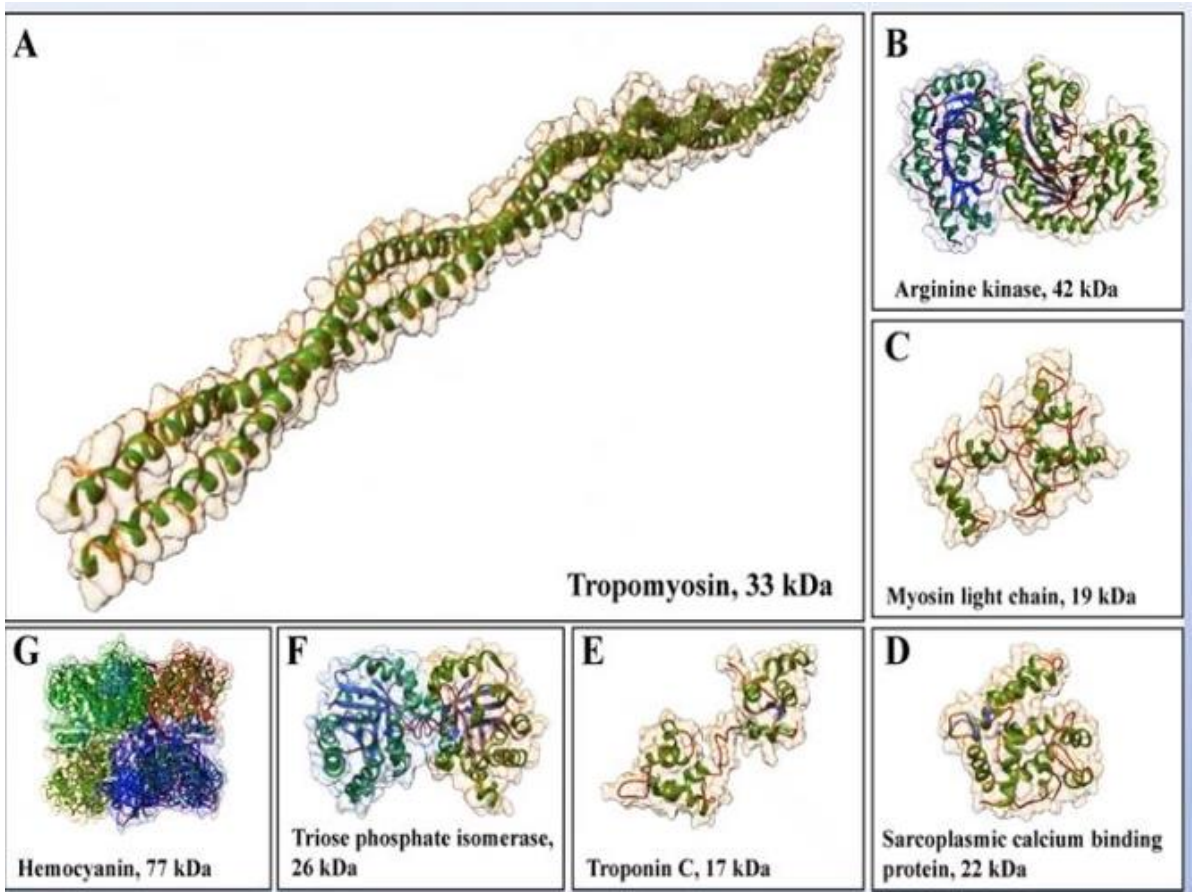


Image: Ruethers et.al, 2018

Predicted novel allergens identified

Protein name	Predicted most similar allergen		Similarity to allergen and resulting predicted evidence for allergenicity		
	Protein allergen	Species	% identity, linear 80 aa window	% identity, 3D epitope	Result
alpha-tubulin	Lep d 33	Lepidoglyphus destructor	98.8	100.0	strong evidence
chitinase	Bla g 12	Blattella germanica	70.0	84.6	weak evidence
heat shock protein 70	Tyr p 28, Der p28	Tyrophagus putrescentiae	92.5 - 98.8	100.0	strong evidence
pyruvate kinase	Sal s 9	Salmo salar	81.2	-	strong evidence
troponin T	Pon l 7	Astacus leptodactylus	93.8	-	strong evidence
vitellogenin	Der f 14	Dermatophagoides farinae	33.8- 37.5	37.5 - 50	weak evidence
glyceraldehyde 3-phosphate dehydrogenase	Per a 13	Periplaneta americana	90.0	100.0	strong evidence
myosin heavy chain	Der f 11	Dermatophagoides farinae	52.5	-	strong evidence

Analyzed by AllerCatPro 2.0. Nguyen et. al,2022

Quantification of Tropomyosin



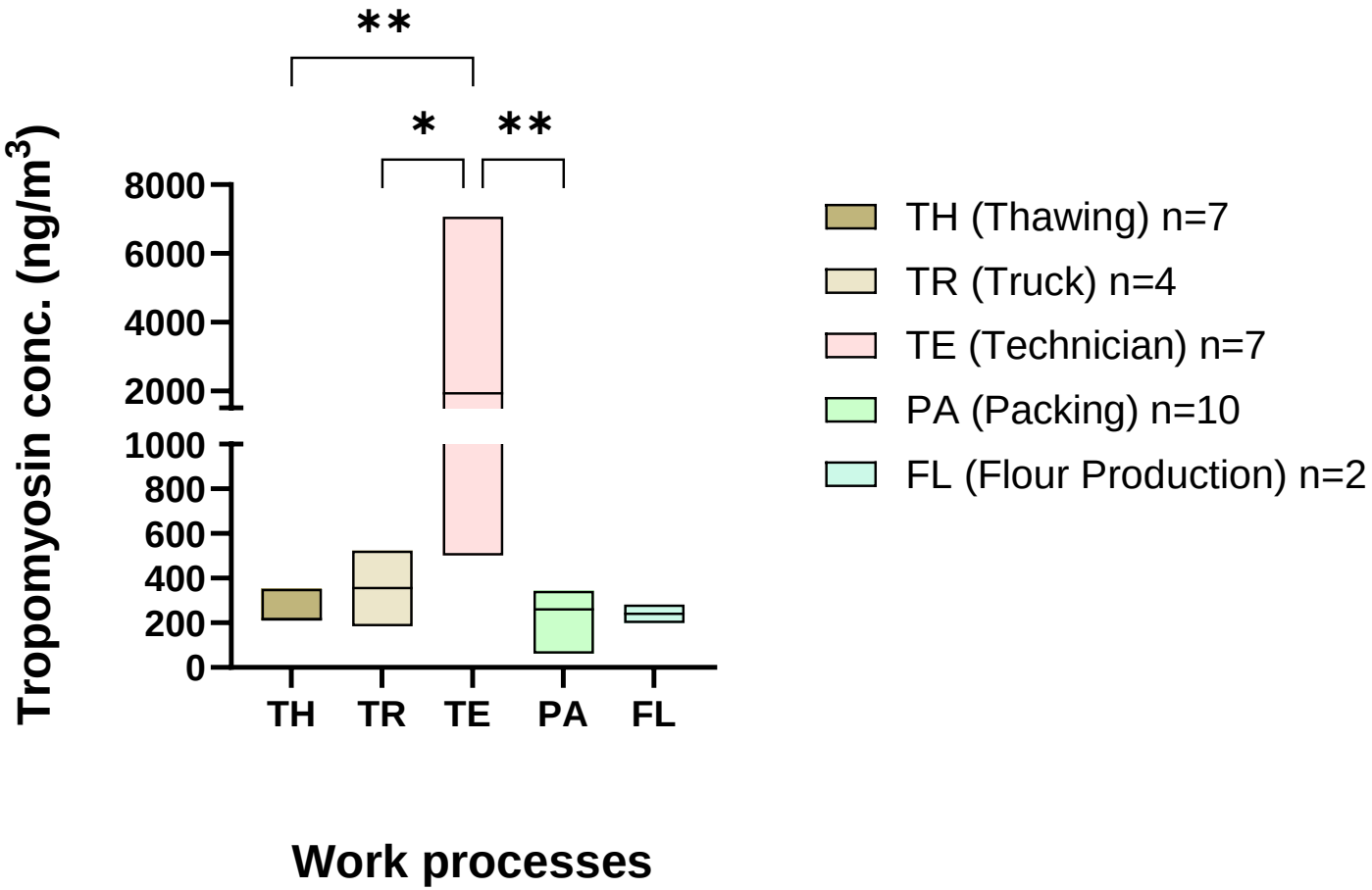
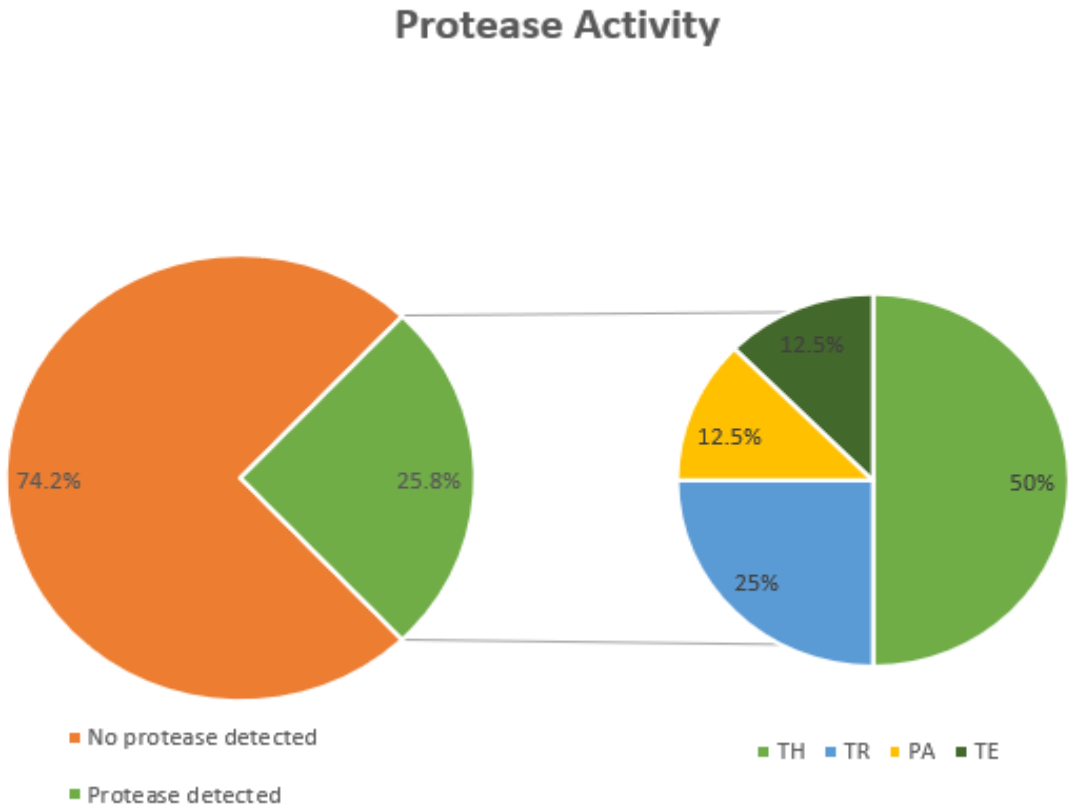


Image: Alamy stock photos

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Detection of protease activity

- Proteases were detected in 25.8 % of the aerosol samples.
- The detected proteases were not consistent with trypsin.
- Metalloprotease were identified in some aerosol samples.



Summary and conclusion

- In this study, we have characterized the allergen exposure and protein composition of bioaerosols in the shrimp industry.
  - Moderately high concentration of total protein.
  - Identified 95 different proteins, including 7 known allergens and 8 potential novel allergens.
  - High concentration of tropomyosin in all work processes.

- The cooking and peeling (Technician) work process is identified as a hot spot for exposure to allergenic proteins and all proteins in general.

- Respiratory symptoms among workers and IgE
  - Very high IgE levels against shrimp and crab were measured in 11% of the exposed workers.
  - There is a high frequency of work-related respiratory symptoms among exposed workers compared to unexposed workers.

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Funding: National Institute of Occupational Health (STAMI)

Thank you for your attention!

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