

Occupational allergy to *Artemia* fish fry feed in aquaculture

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Background	<i>Artemia</i> (brine shrimp) is used as feed for fish fry and shrimp in aquaculture. Two employees in a Norwegian aquaculture research farm reported having chest symptoms when working in an <i>Artemia</i> hatch room.
Aim	To determine the presence and prevalence of <i>Artemia</i> sensitization at the farm and the extent of any <i>Artemia</i> -related respiratory and hand skin symptoms and to identify the allergens involved.
Methods	Participants completed a questionnaire and structured interview. Skin prick tests (SPTs) were performed, and immunoglobulin E (IgE) antibodies to <i>Artemia</i> , shrimp and recombinant tropomyosin were determined. Gel electrophoresis and immunoblots of <i>Artemia</i> extracts were also carried out.
Results	Thirty of 42 employees (71%) participated. Among the 24 subjects exposed to <i>Artemia</i> , four (17%) reported chest and/or hand skin symptoms during exposure and three of them were IgE sensitized to <i>Artemia</i> . Five (21%) of those exposed demonstrated IgE antibodies to <i>Artemia</i> and four (17%) had immediate-positive SPTs. A serum pool from these subjects exhibited IgE binding to a protein of ~97 kDa in the <i>Artemia</i> extract.
Conclusion	Occupational exposure to the <i>Artemia</i> fish fry feed can cause IgE sensitization and allergic symptoms affecting airways and skin.
Key words	Allergy; <i>Artemia</i> ; IgE; occupational; 97 kDa.

Introduction

This study was initiated after two employees reported chest symptoms when working in the hatch room for *Artemia* in a Norwegian aquaculture research farm. The *Artemia* genus, also called brine shrimp (order Anostraca, class Branchiopoda and subphylum Crustacea), is used as feed for aquarium fish and fish fry and shrimp in aquaculture throughout the world.

Crustaceans, mainly of the order Decapoda and class Malacostraca, such as shrimp, crab, crayfish and lobster, are well-known causes of food and occupational allergies [1–5].

The aims of the study were to determine the prevalence of immunoglobulin E (IgE) sensitization to *Artemia* at the research farm, the extent of respiratory and hand skin

symptoms related to *Artemia* exposure and to identify any *Artemia* allergens involved.

Methods

Artemia were stored as cysts at the research farm. For a few weeks two to three times each year, new-hatched cod and halibut fry were fed with living *Artemia*. During these periods, *Artemia* were hatched and fed in separate tanks in the *Artemia* hatch room before being used as food. The *Artemia* tanks contained seawater at 25–27°C. Air was constantly added to the tanks and bubbles induced aerosols. Some employees worked in the hatch room, some fed the fish fry and others joined them in a shift system looking after the whole farm during evenings and weekends.

The entire staff ($N = 42$) were invited to participate in a cross-sectional survey. All participants completed a self-administered questionnaire. One physician, blinded to these questionnaire results, performed a structured interview based on the same questionnaire. The interview gave similar but more extensive information than the questionnaire answers alone and was therefore chosen for further analysis.

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The collected information included demographic data, years employed at the farm, when and where subjects worked with *Artemia*, smoking habits and family history of allergy. Atopy was defined as reported hay fever, a positive skin prick test (SPT) or specific serum IgE to at least one allergen other than *Artemia* or eye and airway symptoms after contact with tree or grass pollens, animals or house dust.

Questions concerning nose and eye symptoms were taken from the International Study of Asthma and Allergies in Childhood questionnaire [6]. In accordance with international recommendations, rhinitis was defined as at least two of the three symptoms of runny nose, blocked nose or itching/sneezing [7]. Questions about chest symptoms (episodic wheezing, dyspnoea or tightness in the chest and cough) were taken from the Norwegian version [8] of the European Community Respiratory Health Survey [9].

Artemia-related symptoms were considered to be present when the symptoms occurred during work in the *Artemia* hatch room or during work with *Artemia* elsewhere but were absent or reduced during weekends or vacations. Symptoms occurring after work shifts were included. *Artemia*-related symptoms were further divided into rhinitis, chest symptoms and hand skin eruptions. Subjects who reported *Artemia*-related symptoms were asked where and when the symptoms first started. If the symptoms had ceased, we explored whether this could be related to reduction or cessation of exposure.

Asthma was defined by answering 'yes' to at least one of the two questions: 'Has a doctor told you you have asthma?' and 'Have you been treated with asthma medicine?' For those with asthma, we noted whether symptoms started before or after the first exposure to *Artemia* and whether any connection to *Artemia*-related chest symptoms was present.

SPTs were performed according to the guidelines of the European Academy of Allergology and Clinical Immunology [10]. Both live *Artemia* directly taken from the hatch tank and *Artemia* stored at -20°C were used for SPT. Subjects with positive SPT reactions to *Artemia* were additionally tested with house dust mite (*Dermatophagoides pteronyssinus*) and shrimp (ALK Abelló, Hørsholm, Denmark). A positive SPT result was defined as a mean duplicate wheal diameter at least 3 mm larger than that of the negative control recorded after 15 min. Positive (histamine hydrochloride 10 mg/ml) and negative controls were supplied by ALK Abelló. SPT was performed by one trained allergy nurse, blinded to the questionnaire results, using a technique that gave a mean histamine wheal diameter of 5.8 mm and a coefficient of variation (standard deviation/mean) for the differences between duplicates ($n = 30$) of 0.13.

Serum IgE and IgE antibodies to recombinant tropomyosin, shrimp, *Artemia*, Phadiatop® (a commercial preparation of timothy, birch, olive and mugwort pollen, *D.*

pteronyssinus, *Dermatophagoides farinae*, *Cladosporium herbarum*, dog and horse allergens) and fx5E® (hen's egg white, cow's milk, cod, wheat, peanut and soybean) were analysed with the CAP-FEIA system (Phadia AB, Uppsala, Sweden). If either of the allergen panels were positive, IgE antibodies to the individual allergens were reanalysed. Serum IgE and IgE antibody levels were defined as elevated if at least 120 kU/l and 0.35 kUA/l, respectively.

Artemia extracts were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunodetection was performed as described previously [11].

IgE reactivities to *Artemia* were analysed by enzyme-linked immunosorbent assay (ELISA) using sera of subjects with positive SPT and IgE to *Artemia* [11].

Results were defined as positive if at least 3% of IgE was bound to a protein in *Artemia* extract and the subject was thereby classified as *Artemia* sensitized.

Statistical analyses were done with SPSS 14.0 for Windows. Median and range were reported for age, employment and *Artemia* exposure.

Informed consent was obtained from all participants. The Regional Committee for Medical Research Ethics for Western Norway (REK Vest) and the Norwegian Data Inspectorate of Medical Research approved the study.

Results

Thirty (71%) employees participated, but two refused to give blood samples for serum IgE analyses. Subjects' characteristics including *Artemia* exposures are shown in Table 1. The majority of participants could be included in more than one of the *Artemia* exposure categories. During the study period, 12 participants worked with *Artemia* and 2 of them in the hatch room. As shown in Table 2, four

Table 1. Characteristics of the 30 participants

	<i>n</i> (%)	Median (years)	Range (years)
Gender: male	14 (47)		
Age		39	22–56
Employment at the farm		7.5	0–26
<i>Artemia</i> exposed	24 (80)		
Worked in hatch room	11 (37)	3.0	0.1–17
Shift work	18 (60)	3.0	0.1–17
Fish fry feeding	18 (60)	5.0	0.1–17
Other situations at the farm	8 (27)	2.5	0.1–13
At previous workplaces	5 (17)	1.0	0.1–2
Not <i>Artemia</i> exposed	6 (20)		
Current smoker	8 (27)		
Never smoked	12 (40)		
Atopy (defined by questionnaire responses)	9 (30)		
Asthma (defined by questionnaire responses)	2 (7)		
Family history of allergy or asthma	16 (53)		

Table 2. Artemia-related symptoms and sensitization among the 24 Artemia-exposed subjects

Subjects	Ever had symptoms			SPT		IgE	
	Chest	Skin	Rhinitis	Immediate (mm)	Late (hours)	ELISA (% bound)	CAP (kUA/l)
A	+	–	–	+ (7)	NR	+ (7)	+ (1.15)
B	–	+	–	+ (11)	NR	+ (11)	–
C	+	+	–	–	+ (11)	+ (23)	–
D	+	–	+	–	NR	–	–
E	–	–	–	+ (8)	NR	+ (77)	–
F	–	–	–	–	NR	+ (5)	–
G	–	–	–	+ (3)	NR	–	–
H	–	–	–	–	+ (24)	–	–
<i>n</i> (%)	3 (13)	2 (8)	1 (4)	4 (17)	2 (8)	5 (21)	1 (4)

Immediate-positive SPT results: wheal diameter (mm); late-positive SPT results: hours from test to appearance of wheal; positive IgE ELISA: at least 3.0% IgE bound to Artemia protein; CAP, ImmunoCap® test system; NR, not reported.

(17%) of the Artemia-exposed subjects reported having ever had Artemia-related symptoms. Subjects A, C and D (Table 2) reported Artemia-related chest symptoms including dyspnoea and cough in all cases and in addition wheeze in Subject C. Subject D reported Artemia-related runny nose and sneezing classified as rhinitis. One of them (Subject A, Table 2) developed doctor-diagnosed asthma after starting work in the hatch room 6 years before the study. Subject E had had childhood asthma.

Positive SPTs to Artemia were seen in 25% (6 of 24) of Artemia-exposed subjects (Table 2). Four of them (subjects A, B, E and G) demonstrated isolated immediate reactions and two subjects (C and H) reported isolated late-phase reactions after 11 and 24 h. In addition, one non-exposed subject reported a late-phase reaction after 48 h. The 26 subjects with immediate negative SPT results had no detectable reaction in the duplicates. All subjects positive to Artemia had negative SPT to shrimp, but subjects E and G demonstrated immediate SPT reactions to house dust mite. Subjects A, B and E had worked in the hatch room.

Table 2 shows that subjects A, B, C, E and F were IgE positive on Artemia ELISA testing; 21 subjects had no detectable IgE binding with this method.

Six of 30 participants (20%) tested positive to common airway allergens (Phadiatop®) and one of them also tested positive to common food allergens (fx5E®). Four subjects had elevated IgE, but none had IgE antibodies to shrimp or recombinant tropomyosin.

Of subjects reporting Artemia-related symptoms, subjects A, B and C, representing 13% of those exposed (Table 2), had IgE antibodies to Artemia (ELISA). They also demonstrated positive SPT: two exhibited immediate reactions and one (Subject C) an isolated late-phase response. Subjects A and C reported that chest symptoms had developed during work in the hatch room between a few months and 1 year after starting work there. They have not had chest symptoms since their work in the hatch

room ended. Subjects B and C had experienced hand skin eruptions during work in the hatch room between 1 and 3 years after starting work there. Subject B's skin symptoms disappeared after changing to a non-exposed job at the farm. Subject C continued to experience itchy hand skin eruptions when handling Artemia outside the hatch room. None of the Artemia-sensitized subjects had developed new Artemia-related symptoms during the last 5 years.

In total, six (25%) of the Artemia-exposed subjects had either an immediate SPT response or a detectable IgE (ELISA) to native Artemia (Table 2); three of them had both, but only Subject A was also positive to the commercial Artemia ImmunoCAP® (Phadia AB). Two of the six (subjects E and G) were also IgE sensitized to common airway allergens (Phadiatop®), including house dust mite and Subject G had elevated IgE. The three subjects who reported Artemia-related symptoms showed evidence of sensitization to Artemia alone. Among those subjects who were neither Artemia sensitized nor reported Artemia-related symptoms, 5 had not been exposed to Artemia and 17 had been, 6 of them also in the hatch room.

Immunoblotting with pooled sera from the six Artemia-sensitized subjects demonstrated major IgE binding to a protein with a molecular mass of ~97 kDa (Figure 1).

Discussion

This cross-sectional study demonstrated that 25% of staff exposed to Artemia fish fry feed were IgE sensitized; four (17%) had experienced Artemia-related symptoms of whom three (13%) reported respiratory symptoms.

A strength of this study is that we used three different methods of detecting Artemia sensitization and with two of them (SPT and ELISA) used native Artemia taken from the hatch tank. IgE sensitization to Artemia was defined by either a positive immediate SPT reaction or the presence of IgE antibodies to Artemia measured by ELISA or ImmunoCAP®. The presence of isolated

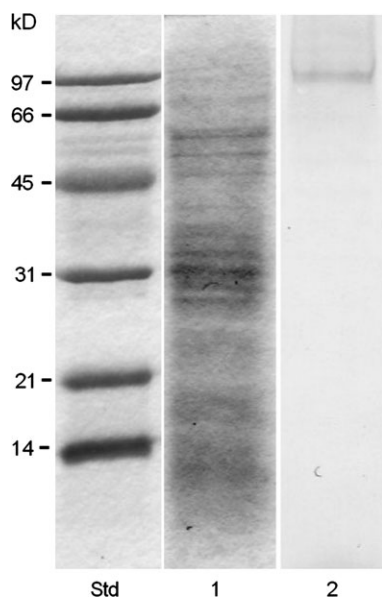


Figure 1. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting of *Artemia* extract. Std, standard molecular weights; 1, SDS-PAGE and 2, blot probe with pooled serum of subjects with positive SPT.

immediate SPT reactions in four subjects is highly suggestive of an IgE-mediated mechanism, not least since in three of these specific IgE antibodies were present in serum. Although the study was not designed to record late-phase SPT reactions, three subjects spontaneously reported infiltration after 11–48 h. The reactions were present in duplicate, were of considerable size and in two cases were also documented photographically. These late reactions were probably caused by immunological mechanisms, either a delayed IgE-mediated reaction (e.g. in Subject E, who had a high ELISA score) or a non-IgE-mediated reaction (Subject H and the non-exposed subject, in both of whom IgE serum antibodies to *Artemia* were not detected).

Subjects IgE sensitized to *Artemia* who reported exposure-related respiratory or skin symptoms were taken to represent cases of clinical allergic reactions to *Artemia*. Using the questionnaire-based definition of chest symptoms probably resulted in high sensitivity but low specificity as an indicator of asthma [12]. Although only Subject A had an asthma diagnosis established by a doctor, Subject C experienced lower airway symptoms, probably of IgE-mediated origin. Hand skin eruptions recorded by the questionnaire may have represented different disease entities, such as protein or allergic contact dermatitis [2,4], as well as allergic contact urticaria. However, since both subjects reporting skin reactions were IgE sensitized to *Artemia*, the latter diagnosis seems more likely. Our symptom questionnaire was not validated clinically, nor were its findings confirmed by exposure or provocation testing, but we think that the use of a standardized questionnaire combined with an interview

strengthened the study since the latter collected more information and provided an opportunity to check whether the questionnaire was fully understood.

Several studies have shown that the risk of becoming sensitized and developing allergic airway symptoms increase with the level of allergen in the breathing atmosphere [2,13,14]. We anticipated the highest allergen concentration to be in the bioaerosols above the hatch and feed tanks, not least since all the reported *Artemia*-related symptoms were related to working there. However, *Artemia* exposure, as either amount of bioaerosol or allergen concentration in the workplace atmosphere, was not monitored in this study. Direct skin contact with *Artemia* and thus the risk of becoming skin sensitized also appeared to be highest in the hatch room since all reported *Artemia*-related skin eruptions first appeared after direct skin contact when working there.

At this particular research farm, most employees worked in the hatch room for a period and then changed to less exposed or non-exposed work tasks without having developed symptoms, but some had become IgE sensitized to *Artemia*. Others changed to lower exposure or unexposed jobs as a consequence of developing *Artemia*-related symptoms. Since symptoms thereby diminished or disappeared, they could continue working at the farm. The healthy worker effect may explain why only one individual reported current *Artemia*-related symptoms and the extent to which previous and non-participating employees had experienced *Artemia*-related allergic manifestations is not known.

We noted that none of the *Artemia*-sensitized subjects had developed new exposure-related symptoms during the previous 5 years. Those who worked in the hatch room during the study period were not *Artemia* sensitized, in spite of having worked there for at least 6 years, the longest exposure times recorded in the study population. This probably reflects different individual genetic dispositions to sensitization.

The study group was relatively small and only six participants (20%) were non-exposed controls. This limited opportunities for statistical analysis of our results and comparison of different exposure and symptom groups.

The cross-sectional design of this study does not allow confirmation of a causal relationship between occupational exposure, sensitization and clinical symptoms. In order to confirm the consequences of occupational exposure to *Artemia*, a longitudinal study is required.

The 97-kDa IgE-binding protein from the *Artemia* extract may be identical to paramyosin, an invertebrate muscle protein. Allergy to crustacean paramyosin is uncommon, and where it is implicated as an allergen other proteins, especially tropomyosin, seem to be more heavily involved [15,16]. Paramyosin is also one of the proteins binding IgE in subjects allergic to the fish parasite *Anisakis simplex* [17] and is one of the IgE-binding proteins in mite extracts [18].

To our knowledge, only two case histories of Artemia allergy have been published to date. In one, a technician at a research farm producing fish food containing Artemia and shrimp developed Artemia-related allergic rhinoconjunctivitis and asthma [19] and in the other probable allergic conjunctivitis occurred in an atopic individual using Artemia to feed fish in his hobby aquarium [20]. The exposure conditions in these cases were different from those in our study.

The results from this study support these two case histories in suggesting that Artemia can sensitize and elicit allergic symptoms in exposed workers. In this study population, the ImmunoCap® test system showed low sensitivity compared to ELISA and SPT. In 1997, >1500 metric tons of Artemia cysts were used in shrimp and fish hatchery industries [21] and the farm in this study uses 50–100 kg Artemia cysts each year. We believe the potential of Artemia as an occupational allergen in the widespread and rapidly growing aquaculture research and farming industry needs more extensive evaluation. Further studies should include larger exposed populations and should involve clinical validation of questionnaire-based allergic symptom reports, monitoring of Artemia exposure and documenting of the relationship between exposure and clinical symptoms by provocation or exposure tests. At this farm, the ventilatory system and the procedures for handling Artemia, including the use of gloves, have been improved in the hatch room following this study. Further studies on the Artemia allergen are in progress.

Key points

- Artemia is used as feed for aquarium fish and fish fry and shrimp in aquaculture throughout the world.
- Working with Artemia fish fry feed in aquaculture can cause occupational respiratory and skin allergy, probably with the highest risk when hatching and feeding Artemia.
- Not all individuals with Artemia allergy demonstrate specific serum IgE sensitization to the commercial ImmunoCap® test system.

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Conflict of interest

None declared.

References

1. Lehrer SB, Ayusa R, Reese G. Current understanding of food allergens. *Ann N Y Acad Sci* 2002;**964**:69–85.
2. Jeebhay MF, Robins TG, Lehrer SB, Lopata AL. Occupational seafood allergy: a review. *Occup Environ Med* 2001;**58**:553–562.
3. Ortega HG, Daroowalla F, Petsonk EL *et al.* Respiratory symptoms among crab processing workers in Alaska: epidemiological and environmental assessment. *Am J Ind Med* 2001;**39**:598–607.
4. Schärer L, Hafner J, Wüthrich B, Bucher C. Occupational protein contact dermatitis from shrimp. *Contact Dermatitis* 2002;**46**:181–182.
5. Lehrer SB, Ayuso R, Reese G. Seafood allergy and allergens: a review. *Mar Biotechnol* 2003;**5**:339–348.
6. Asher MI, Keil U, Anderson HR *et al.* International study of asthma and allergies in childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;**8**:483–491.
7. International Rhinitis Management Working Group. International consensus report on diagnosis and management of rhinitis. *Allergy* 1994;**49**(Suppl. 19):5–34.
8. Svanes C, Omenaas E, Eide GE, Fluge Ø, Gulsvik A. Hospitalisation for lung disease in early childhood and asthma symptoms in young adulthood. *Respir Med* 1998;**92**:1003–1009.
9. Burney PGJ, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994;**7**:954–960.
10. European Academy of Allergology and Clinical Immunology. Allergen standardisation and skin tests. Position paper. *Allergy* 1993;**48**(Suppl. 14):48–82.
11. Van Do T, Elsayed S, Florvaag E, Hordvik I, Endresen C. Allergy to fish parvalbumins: studies on the cross-reactivity of allergens from 9 commonly consumed fish. *J Allergy Clin Immunol* 2005;**116**:1314–1320.
12. Becklake MR, Malo J-L, Chan-Yeung M. Epidemiological approaches in occupational asthma. In: Bernstein IL, Chan-Yeung M, Malo J-L, Bernstein DI, eds. *Asthma in the Workplace*. 2nd edn. New York: Marcel Dekker Inc, 1999.
13. Heederik D, Doekes G, Nieuwenhuijsen MJ. Exposure assessment of high molecular weight sensitizers: contribution to occupational epidemiology and disease prevention. *Occup Environ Med* 1999;**56**:735–741.
14. Nieuwenhuijsen MJ, Putcha V, Gordon S *et al.* Exposure-response relation among laboratory animal workers exposed to rats. *Occup Environ Med* 2003;**60**:104–108.
15. Griffin P, Allan L, Gibson M, Elms J, Wiley K, Curran AD. Measurement of personal exposure to aerosols of *Nephrops norvegicus* (Scampi) using a monoclonal-based assay. *Clin Exp Allergy* 2001;**31**:928–933.

16. Asero R, Mistrello G, Roncarolo D, Amato S. A case of allergy to airborne, heat labile shrimp allergens. *J Allergy Clin Immunol* 2002;**109**:371–372.
17. Perez-Perez J, Fernandez-Caldas E, Maranon F *et al.* Molecular cloning of paramyosin a new allergen of *Anisakis simplex*. *Int Arch Allergy Immunol* 2000;**123**:120–129.
18. Tsai LC, Peng HJ, Lee CS, Chao PL *et al.* Molecular cloning and characterization of full-length cDNAs encoding a novel high-molecular-weight *Dermatophagoides pteronyssinus* mite allergen, Der p 11. *Allergy* 2005;**60**:927–937.
19. Carino M, Elia G, Molinini R, Nuzzaco A, Ambrosi L. Shrimp-meal asthma in the aquaculture industry. *Med Lav* 1985;**76**:471–475.
20. Moscato G, Vinci G, Pisati A *et al.* Allergia da acquario: un caso di congiuntivite isolata da *Artemia* adulta e *Chironomus thummi*. *Giorn. It.Allergol.Immunol. Clin* 1991;**1**:141–145.
21. Lavens P, Sorgeloos P. The history, present status and prospects of the availability of artemia cysts for aquaculture. *Aquaculture* 2000;**181**:397–403.