# ALLERGY AND ASTHMA TO INDIGENOUS SEAFOOD SPECIES IN SOUTH AFRICA

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## ABSTRACT

Worldwide, food allergies and anaphylaxis are increasing at a faster rate than any other allergic disorders, affecting up to 4% of adults and 8% of children.[1] Most allergic reactions to foods can be attributed to a few food groups including seafood, which form important protein sources in Africa. The move to healthier eating habits that promote seafood consumption has resulted in more frequent reporting of allergic health problems among consumers as well as processors of seafood.[2,3] Increased efforts aimed at developing more specific diagnostic tests as well as effective therapies and preventive measures are dependent on the detailed characterisation of seafood allergens. Over the past decade, a number of studies of allergic disease outcomes associated with indigenous seafood species have been conducted among consumers and processors in South Africa. These studies have focused on identifying and characterising the implicated allergens using a combination of epidemiological, immunological and molecular approaches.

## SEAFOOD HARVESTED IN SOUTH AFRICA AND AGENTS IMPLICATED IN ADVERSE REACTIONS

In 2003, the total harvest of seafood in South Africa amounted to 673 812 tons of which about 80% was exported with a wholesale value of US$ 4.8 million.[4,5] Bony fish such as anchovy, hake and pilchard continue to be the most commonly processed seafood followed by crustaceans (e.g. rock lobster). This was also demonstrated in our postal survey of workplaces along the West Coast of South Africa in which 76% of all seafood processing workplaces processed bony fish and 34% processed rock lobster.[6] The major seafood groups that induce allergic reactions belong to three phyla[7] including bony fish, crustacean and molluscs (see Table I). The two invertebrate phyla of crustaceans and molluscs are generally referred to as 'shellfish'.

The allergic and inflammatory reactions associated with seafood experienced by consumers and workers in the seafood processing industry are the result of exposure to the seafood and its various components, all capable of causing adverse reactions (muscle and connective tissue, exoskeleton, blood, fish juice, skin, skin slime/mucin, gut – including enzymes such as pepsin and trypsin).[2] The major allergens responsible for ingestion-related allergic reactions due to fish are the parvalbumins[7] and tropomyosin in crustaceans[8] while molluscs contain various less well characterised allergens, as we have demonstrated.[9,10]

The most comprehensive study on a seafood allergen has been the analysis of the allergen parvalbumin from codfish, Gad c 1. Since this 12 kDa calcium-binding muscle protein shares about 60-80% amino-acid homology with similar proteins from hake and carp, this may explain some degree of cross-reactivity observed in fish-allergic patients. However, clinical reactivity to various fish species may differ and other as yet uncharacterised seafood allergens could also be of clinical significance. This is particularly important for aerosolised allergens that are inhaled by consumers during food preparation or by workers in the food-processing industry. Current investigations in our laboratory characterised the molecular structure of pilchard (Fig. 1) parvalbumin in detail[11] and demonstrated the effect of processing on the allergenicity of these allergens.[12]

The major heat-stable allergen in most investigated crustacean species is tropomyosin which belongs to a family of highly conserved structural proteins found in muscle tissue.[8] Over 30 000 living crustacean species are found worldwide (Fig. 2) and large varieties are consumed either raw or cooked. Interestingly, crustaceans

*Fig. 1. The most common fish species processed in South Africa, pilchard,* Sardinops sagax *(www.fishbase.org).*

*Fig. 2. The most common crustacean species processed in South Africa, West Coast rock lobster,* Jasus lalandii *(Courtesy Prof Griffith, UCT).*

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are classified as arthropods together with spiders and insects.[13,14] Not surprisingly, allergenic tropomyosins have also been identified in insects such as cockroaches,[15] house-dust mites and locusts,[16] having possible clinical significance because of their cross-reactivity. Furthermore, we have demonstrated for different South African crustacean species that IgE antibody-binding profiles vary among patients and that besides tropomyosin other, as yet unidentified, allergens are implicated.[8,10]

The group of molluscs, subdivided into the classes Bivalvia, Cephalopoda, and Gastropods (Table I) comprises over 100 000 different species, including several important seafood groups such as mussels, oysters, abalone, snails, and squid (calamari). We identified the major allergens from abalone [9] (Fig. 3) with molecular weights of 38 kDa and 49 kDa (Hal m 1), suggesting tropomyosin as being one of these allergens (Fig. 4). Using immunoblot analysis, patients sensitised to abalone also demonstrated the complexity of immunological reactions to other mollusc species as well as to house-dust mite (Fig. 5).

***Table I. Classification of seafood groups causing allergic reactions***

| Phylum | Class | Common name |
|---|---|---|
| **Mollusca** | Gastropoda | Abalone, snail, limpet, |
| | Bivalvia | Clam, mussel, oyster, scallop |
| | Cephalopoda | Octopus, squid |
| **Arthropoda** | Crustacea | Rock lobster, crab, shrimp, crayfish |
| **Chordata** | Osteichthyes | Pilchard, cod, hake, salmon, anchovy, tuna, mackerel, yellowtail |

Among consumers the consumption of raw or insufficiently processed seafood poses probably the greatest risk to human health. Allergy-like symptoms, particularly after consumption of fish, are often caused by end products of bacterial activity causing so-called scombroid toxication.[7,17] Fish with high concentrations of the amino acid histidine, such as yellowtail and snoek, are particularly problematic, as highlighted in our recent survey among food outlets in South Africa.[18] Marine biotoxins, generated by algae, are found in fish as well as in mussels and abalone. These biotoxins can also cause allergy-like symptoms and are, as all true food allergens, not destroyed by cooking.[7] In addition, contaminants such as nematode parasites (e.g. *Anisakis pegreffii*) have recently been reported to be allergenic among seafood-processing workers in South Africa.[19,20]

*Fig. 3. Abalone species from various parts of the world.*

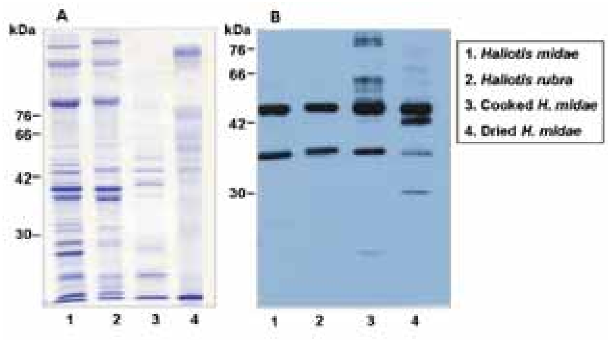


*Fig. 4. A. SDS-gel electrophoresis of four different abalone extracts: South African abalone* Haliotis midae *(1), Japanese abalone* Haliotis rubra *(2), cooked* H. midae *(3) and dried* H. midae *(4). B. Western blot of serum IgE reactivity of abalone-sensitive patient to the identical extracts.*

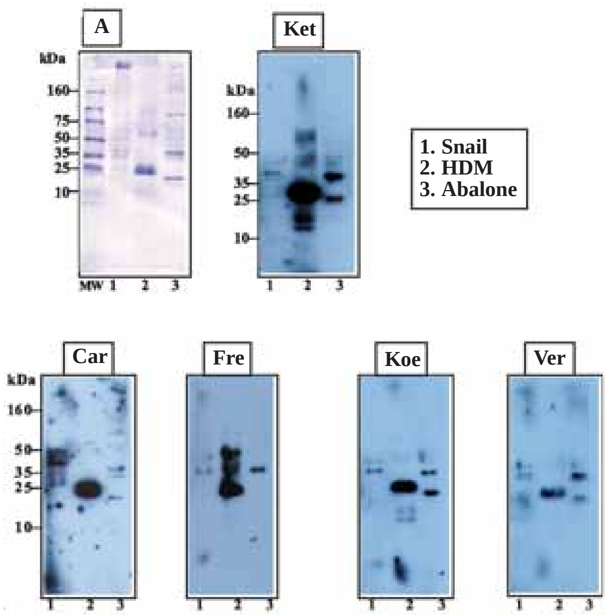


*Fig. 5. Western blot of serum IgE reactivity of abalone-sensitive patients to extracts of snail, house-dust mite (HDM) and abalone. One representative blotting membrane stained with Coomassie Blue is displayed in A. The species names are indicated from 1 to 3. The molecular weights (MW) are indicated on the left side in kilodalton (kDa).*

## STUDIES OF ALLERGY TO SEAFOOD SPECIES AMONG SOUTH AFRICAN CONSUMERS

Our study conducted in the Western Cape found that over 50% of the allergic reactions seen among 105 sensitised individuals were to different crustacean species, followed by 30% to molluscs and 20% to fish.[10,21] More than half of the individuals reacted to one seafood group, 36% to two seafood groups and surprisingly only 11% to all three seafood groups. Among the mollusc-sensitive group almost 50% reacted to abalone, which subsequently resulted in the identification and characterisation of a novel allergen by our group.[9]

A follow-up study in our clinic confirmed that avoidance of ingestion of crustacean does not necessarily protect patients from allergic reactions, as they may be accidentally exposed through other means. Six patients investigated over a period of 4 years demonstrated that

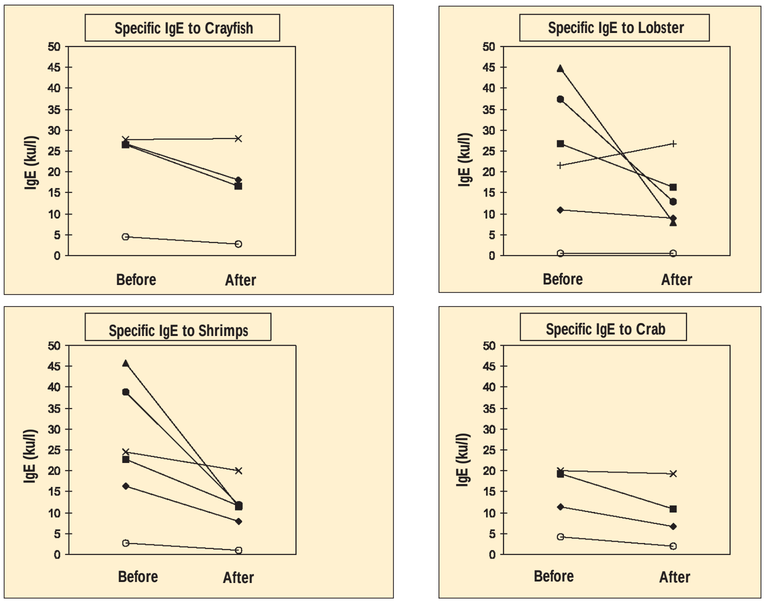


*Fig. 6. Specific IgE reactivity of six crustacean-sensitive patients 'before' consulting our clinic and 'after' 4 years as determined by ImmunoCAP in kU/l. Most subjects demonstrated a decrease in IgE reactivity to the four crustacean species. Note that all IgE levels were still significantly elevated after 4 years of avoiding ingestion of crustaceans.*

specific IgE levels were still significantly elevated (Fig. 6),[10] indicating the long half-lives of these antibodies and possible stimulation of the immune system by other cross-reactive allergens, such as house-dust mites (see Fig. 5).

Furthermore, infection and allergic sensitisation with the fish parasite *Anisakis* have frequently been reported worldwide following consumption of undercooked fish, indicating that allergic reactions can be falsely diagnosed as fish allergy.[19,20] This has been supported by our epidemiological study of workers from the fishing village on the West Coast of South Africa in which 5% reported allergic symptoms to seafood with the majority reporting symptoms after eating (87%), touching (40%) or smelling (17%) seafood. Analysis of fish consumption patterns among the entire workforce studied, revealed that workers with high seafood consumption of fish (as measured by serum levels greater than 2.23 % wt/wt of 20:5 n-3 omega-3 fatty acid versus <1.28 weight % of 20:5 n-3 omega-3 fatty acid), had a twofold increased risk (odds ratio (OR) = 2.24) of being sensitised to *Anisakis* but not to fish, indicating this parasite is an important allergen.[19,20]

*Fig. 7. Fish packers sorting pilchard for degutting and canning, resulting in exposure to allergens via inhalation and skin exposure.*

## STUDIES AMONG WORKERS PROCESSING SEAFOOD FROM SOUTH AFRICAN WATERS

The seafood industry in South Africa employs approximately 30 000 workers in direct employment in more than 100 workplaces and 60 000 workers in related jobs, supplying food for the entire Southern African subregion.[2,3,6,22] Labour in this industry tends to be divided along gender lines with men almost exclusively going out to sea to catch the fish and women doing the majority of on-land processing. A large proportion (62%) of the workforce in fish-processing plants is female and at least one-third of the workforce is employed on a seasonal basis by the industry. The workers are involved predominantly in bony-fish (pilchard, anchovy and hake) processing activities.

The study investigating the risk of occupational allergy associated with pilchard and anchovy processing on the West Coast of South Africa found that workers were at substantial risk of inhaling aerosols containing fish antigens that resulted in an increased risk of developing occupational asthma[23] (Fig. 7). High fish-antigen levels were encountered during fishmeal production and bagging activities ranging from 81 to 75 748 ng/m$^3$.[24] A high correlation was found between ambient pilchard- and anchovy-antigen concentrations (Pearson r = 0.71, $p < 0.001$).

The study also found that workers were at risk of developing occupational allergies, with rhinoconjunctivitis (2.6%) being more prevalent than asthma (1.8%) and protein contact dermatitis or urticaria (1-2%).[25] A dose-response relationship was demonstrated between the ambient level of fish antigen exposure at the time of symptom onset and the risk of such work-related asthma symptoms. Workers with work-related asthma symptoms had a twofold increased risk of being exposed to pilchard-antigen concentrations > 30 ng/m$^3$ at the time of onset of new symptoms (OR: 2.25, confidence interval (CI): 1.06-4.75). These workers were working mainly in the fishmeal processing/bagging and fish canning departments. In respect of skin problems, workers with recurrent skin symptoms were more likely to be involved in canning/jetty activities, associated with high incidence of skin contact with fish and wet fish products.[26] These workers were also more likely to have positive skin reactivity to fish on skin-prick tests.

Furthermore, sensitisation to the fish parasite *Anisakis* was high (8%), with *Anisakis*-specific IgE reactivity being strongly associated with protein contact dermatitis and bronchial hyperreactivity. A detailed analysis of the underlying immunological mechanisms has recently been published.[19,20]

The findings of these studies will add to the growing body of evidence on occupational allergy among fish-processing workers.

## MURINE MODELS OF ALLERGY TO SEAFOOD AND ASSOCIATED ALLERGENS FOUND IN SOUTH AFRICA

To explain the pathogenetic mechanisms responsible for the increased risk of allergy and asthma to seafood and associated agents in seafood workers, we developed various murine models.

While many parasites are believed to protect against allergy, the fish worm *Anisakis* is known to cause acute gastro-allergic or anaphylactic reactions in sensitised patients. An experimental murine model of sensitisation to the fish parasite *Anisakis* was developed as this parasite commonly infests more than 80% of fish harvested in Southern African waters. In this murine model, infection with live *Anisakis* strongly increased Th2/type 2 immunological responses and predisposed mice to allergic reactions upon oral challenge with soluble *Anisakis* proteins.[19,20] Subsequently, we could also demonstrate that skin[27] and nasal exposure[28] to *Anisakis* allergens is able to elicit airway hyperresponsiveness in a murine model. Drawing these findings together, we could demonstrate the immunobiological basis for the increased risk of sensitisation to *Anisakis* due to contaminated seafood in the occupational and domestic environment.

Subsequently a murine model was also developed to investigate sensitisation to allergens in pilchard, the major fish species processed in the factories previously investigated. Sensitisation models with purified natural parvalbumin from pilchard and comparative studies with recombinant parvalbumin from carp strikingly demonstrated IgE-mediated sensitisation and the development of airway hyperresponsiveness on exposure to these allergens.[29] This indicates that the route of exposure and immunological activity of specific seafood allergens can be studied in great detail using murine models.

## APPLICATION OF RESEARCH FINDINGS LOCALLY AND INTERNATIONALLY

The findings of our different research approaches on seafood exposure and health effects have directed practical applications in various different areas.

### *Detection of seafood presence in various products*

The detection and identification of the origin of seafood tissue has been utilised in the forensic area to trace poaching activities on South African abalone [30] (see Fig. 4) and can assist in the management and conservation of marine organisms.[31] The DNA-based technologies have been supplemented by robust monoclonal antibody-based assays, which allow the differentiation of abalone tissue from other molluscs in less than 1 hour.[32] The techniques developed will be important in the detection of allergenic seafood tissue from unidentified food sources, which is critical for preventing allergenic cross-contamination problems in the food industry, as has been recently demonstrated.[33]

### *Detection of toxins in seafood*

Allergy-like reactions can be caused by contamination of seafood with unidentified components such as nematodes, which can not only cause infection with the live parasites but also severe allergic reactions as demonstrated in our occupational studies.[2,19,20,24,25,34] Furthermore, the detection of contamination of seafood products with toxins, generated by algae[7,17] or bacteria,[18] is vital for correct diagnosis, as the symptoms experienced in reaction to these toxins are very similar to those to allergenic proteins. This issue has been further highlighted by the endocrine-disrupting effects observed in male swordfish species from Southern Africa and Europe, raising concern regarding the contamination of local waters with xenobiotic chemicals due to long-term use of toxic pesticides.[35]

### *Evaluating environmental exposure in seafood-processing workplaces*

Aerosolisation of seafood allergens has been identified as a route for allergic sensitisation in workers of the seafood processing industry (see Fig. 7) and also among consumers during the preparation of seafood during cooking.[21,23-25] We developed sensitive immunological techniques to detect exposure to aerosolised fish allergens.[24,36] These techniques are currently being extended among international investigators to evaluate airborne exposures in other seafood groups such as the crustaceans and molluscs to address the major industries in other countries, e.g. Australia.[37,38] Another application of these assays is the evaluation of interventions in the workplace to reduce the incidence of occupational allergies and asthma due to airborne exposures.[39]

### *Improved diagnosis and treatment approaches*

From our murine model studies we have been able to show that the generation of purified and recombinant parvalbumin from pilchard will assist in the sensitive diagnosis of sensitisation to this major allergen.[11,12] This has other potential applications including assisting in the development of safe and specific immunotherapeutic approaches for fish-allergic individuals.[40] Detailed work in this area is currently in progress.

## FUTURE RESEARCH DIRECTIONS

Our studies of seafood consumers and workers in South Africa in the past decade have contributed towards a tremendous wealth of information regarding the adverse health effects and allergic experiences of these groups. These findings have created opportunities for clinical and technological applications in an effort to prevent allergy and asthma due to ingestion, skin contact or inhalation of seafood. However, it has also raised a number of new questions that need further investigation. Future research needs to be directed towards the detailed characterisation of these seafood allergens using state-of-the-art immunological, biochemical and molecular techniques. This will contribute to the development of more sensitive and specific diagnostic techniques for evaluating affected individuals. Furthermore, there is a need for the development of more user-friendly methods for the environmental detection of seafood allergens that have more widespread application in different settings such as contamination of food matrix for consumers as well as food-processing work environments. The influence of other possible contributory factors to allergic responses, such as marine biotoxins, endotoxins and parasites, needs closer investigation. In particular, the role of cross-reactive pan-allergens such as tropomyosin found in ingested seafood and inhaled insect and mite allergens, warrants further scrutiny.

### Declaration of conflict of interest

The authors declare no conflict of interest.

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