Tropical Biomedicine 22(2): 171–177 (2005)

Identification of major allergens of two species of local snappers: *Lutjanus argentimaculatus* (merah/ red snapper) and *Lutjanus johnii* (jenahak/ golden snapper)

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Abstract. Fish has been recognized as a source of potent allergens both in food and occupational allergy. *Lutjanus argentimaculatus* (red snapper) and *Lutjanus johnii* (golden snapper) locally known as merah and jenahak, respectively, are among the most commonly consumed fish in Malaysia. The objective of this study is to identify the IgE-binding proteins and major allergens of these species of fishes. Extracts of both fish species were prepared and fractionated by sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). IgE binding patterns were then demonstrated by immunoblotting using sera from patients allergic to the fishes. The raw extracts of both fish produced 26 protein bands. Both species of fishes had similar protein profiles. In cooked extracts, several protein bands in the range of about 40 to 90 kD which were present in the uncooked extracts appeared to be denatured and formed high molecular weight complexes. The immunoblotting of golden snapper and red snapper revealed 16 and 15 various IgE-binding bands, in the range of 151 to 12-11 kD, respectively. A 51 kD protein was identified as a major allergen for both fishes. A 46 kD protein was also demonstrated as a major allergen in golden snapper and a 42 kD protein was also seen as a major allergen in red snapper. A heat-resistant protein of ~12 kD which is equivalent in size with fish parvalbumin was demonstrated only as minor allergen for both fishes.

INTRODUCTION

Fish allergy is frequently encountered in coastal areas where the consumption and processing of fish are common (James *et al.*, 1997). The clinical symptoms of fish allergy includes urticaria, angioedema, asthma, rhinitis, vomiting, diarrhoea and anaphylaxis (Hamada *et al.*, 2003b; Lopata & Potter, 2001).

Although fish contains a wide variety of proteins, only a few are known allergens (Jeebhay *et al.*, 2001). The main allergen in fish, Gad c 1, a 12 kD protein from codfish was the first fish allergen to be isolated, characterized and identified as parvalbumin (Lopata & Potter, 2001). Parvalbumin is a family of calcium-binding proteins of muscle tissue which play an important role in muscle physiology (Hamada *et al.*, 2003b). Subsequently, other fish allergens have been characterized from salmon *Sal s* 1 (Van Do *et al.*, 1999), carp *Cyp c* 1 (Bugajska-Scheretter *et al.*, 2000), cod Baltic *Gad m* 1 (Das Dores *et al.*, 2002a) and mackerel *Sco j* 1, *Sco a* 1 and *Sco s* 1 (Hamada *et al.*, 2003b). Recently, fish collagen (~100 kD) has been identified as a fish allergen and found to be a highly cross-reactive allergen among various species of fish (Hamada *et al.*, 2001; Hamada *et al.*, 2003a). Aldehyde phosphate dehydrogenase (APDH), a ~41 kD allergen from codfish has also been reported as a fish allergen (Das Dores *et al.*, 2002b).

Fish from the Lutjanidae family are widely consumed in Malaysia. The genus *Lutjanus* commonly known as snapper is represented by 103 species worldwide. Of these, 34 species have been documented in Malaysia. These fishes are among the

dominant species caught, comprising about 16% of the total local catch (Mohsin & Ambak, 1996). Two common species available for local consumption are merah/ red snapper (*Lutjanus argentimaculatus*) and jenahak/ golden snapper (*Lutjanus johnii*). To date, information on allergy to snappers is very limited. At present, the only report on snapper allergy was carried out and a protein of 12.5 kD which corresponds to fish parvalbumin was described as major allergen of the fish (James *et al.*, 1997). Major allergens generally are defined as proteins for which 50% or more of the allergenic patients studied have specific IgE (Helfe, 1996). The aim of this study is to identify the IgE-binding proteins and major allergens of two species of local snappers.

MATERIALS AND METHODS

Allergen Extracts

Raw and cooked extracts of both fishes were prepared according to the methods described elsewhere (James *et al.*, 1997; Yamada *et al.*, 1999). In brief, the fish meat was homogenized with 0.1M phosphate buffered saline pH 7.2 (PBS) at a 20% wt/vol solution, followed by overnight extraction at 4°C with constant mixing. The homogenates were then centrifuged, filtered and lyophilized.

SDS-PAGE (Sodium Dodecyl Polyacrylamide Gel Electrophoresis)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using a method described by various studies (Hansen *et al.*, 1997; James *et al.*, 1997; Yamada *et al.*, 1999). The fish extracts were heated in SDS reducing buffer. Each sample and the prestained molecular weight markers were electrophoresed in a 12.5% separating gel with a 5% stacking gel. Gels were stained with Coomassie Brilliant Blue R-250 (BioRad, USA).

Immunoblotting and Detection of Specific IgE

IgE-binding components were determined using sera from 40 and 32 patients with fish allergy and positive SPT to golden and red snapper, respectively. Serum from a non-allergic individual was used as a negative control. The western blot was performed following the procedure described by Bernhisel-Broadbent *et al.* (1992). After SDS-PAGE, proteins in the unstained gel were transferred to 0.45 µm nitrocellulose membrane. After immunoblotting, the membrane was cut into strips and then blocked in 10% non-fat milk in tris- buffered saline (TBS). The membrane was incubated with sera overnight at 4°C under constant mixing. The membrane was then incubated in biotinylated goat antihuman IgE (Kirkergaard and Perry Laboratories, UK) for 30 minutes, followed by incubation in streptavidin-conjugated alkaline phosphatase for 30 minutes (BioRad, USA). The Alkaline Phosphatase Conjugate Substrate kit (BioRad, USA) was used to detect the bound IgE.

RESULTS

Molecular Weight of Fish Proteins

Figure 1 shows the coomassie-stained protein bands of golden snapper and red snapper. The raw extracts of both fish species produced approximately 26 protein bands between >175 to ~12 kD. All bands were common to both species of fish. Several bands of red snapper extract were very faint in the coomassie-stained gel. Cooked extract of both fish produced fewer protein bands compared to the raw fish extracts. Several proteins in the cooked extract appeared to be denatured in the range of about 40 to 90 kD, and formed high molecular weight (>100 kD) complexes. The cooked extract of golden snapper demonstrated 14 bands, while red snapper had only 11 bands. A ~12 kD protein band which is most likely to be fish parvalbumin was detected in all extracts.

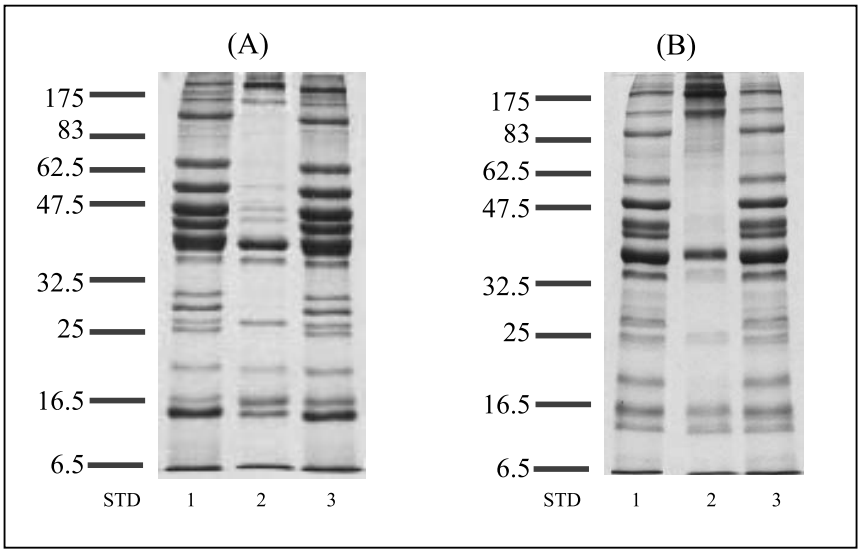


Figure 1. Coomasie blue-stained SDS-PAGE gel of extracts of golden snapper (A) and red snapper (B). Lane 1, raw extract; lane 2, cooked extract. STD, molecular weight marker in kilo Dalton (kD).

Determination of IgE-Binding Proteins

Figure 2 and table 1 and 2 show IgE-binding pattern of golden snapper and red snapper. The sera possessed IgE-antibodies specific to various fish-protein fractions in the range of 151 to 12-11 kD. IgE-immunoblotting revealed 16 and 15 specific IgE-binding proteins for golden snapper and red snapper, respectively. A 51 kD protein was identified as a major allergen for both fish species. A 46 kD protein was also demonstrated as a major allergen in golden snapper and a 42 kD protein was also seen as a major allergen in red snapper. Both fish species demonstrated two more heat-labile allergens at 70 and 60 kD.

Several heat-resistant proteins of higher and lower molecular weights have also been demonstrated as minor allergens. Heat-resistant protein at ~12 kD which is equivalent in size with fish parvalbumin was detected as minor allergen in both fish species. Similarly, high molecular weight heat-resistant proteins at 151, 125 and 90 kD have also been demonstrated as minor allergens in both, but more prominent in red snapper.

No IgE-binding was observed with control sera in immunoblotting of both fish species (figure 2).

DISCUSSION

Fish constitute one of the main causes of allergic reactions in food allergy as well as in occupational allergy. In a previous study, we found that the prevalence of fish and shellfish allergy to be 44% among patients with allergic rhinitis (Shahnaz *et al.*, 2001). This is not surprising as fish is a major component of the local diet. For this study we selected two species of local snappers of the *Lutjanus* spp.

Fish generally contains a wide variety of proteins and IgE-binding allergens of various molecular weights (Bernhisel-Broadbent *et al.*, 1992; Hansen *et al.*, 1997). We found that both fish species had a protein profile of 26 protein bands ranging in molecular weight from ~12 to >175 kD, and immunoblotting revealed that several bands ranging from ~12 to 151 kD were able to bind specific IgE from the sera of fish-sensitized patients.

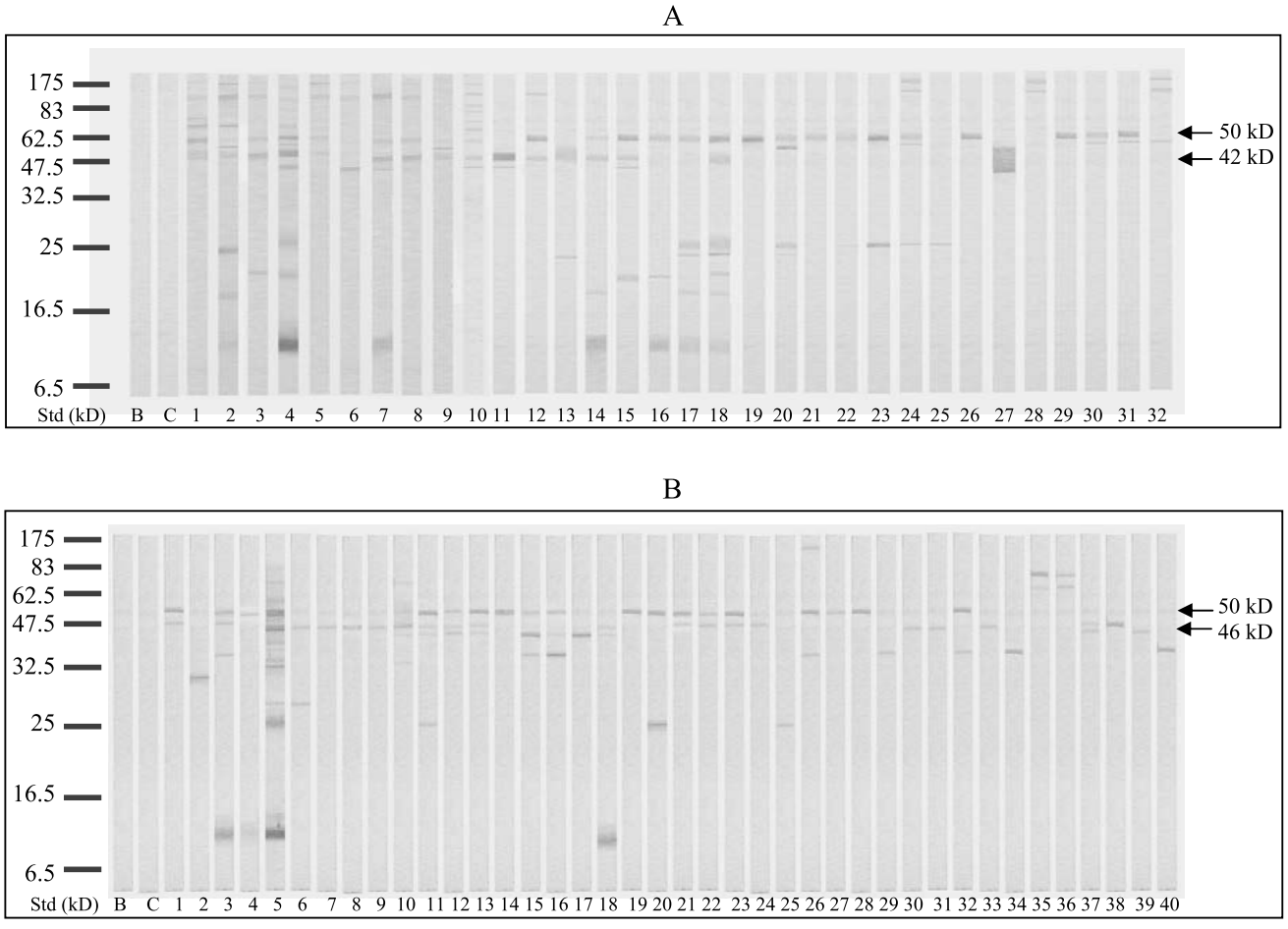


Figure 2. Immunoblot of red snapper (A) and golden snapper (B) extracts demonstrating IgE-binding proteins. Lane Std is molecular weight marker in kiloDalton (kD). Lanes B and C are blank and negative control, respectively. Lanes 1 to 32 in A and lanes 1 to 40 are blots incubated with different serum samples.

Table 1. The frequency of specific-IgE binding proteins in 32 patients sensitized to red snapper

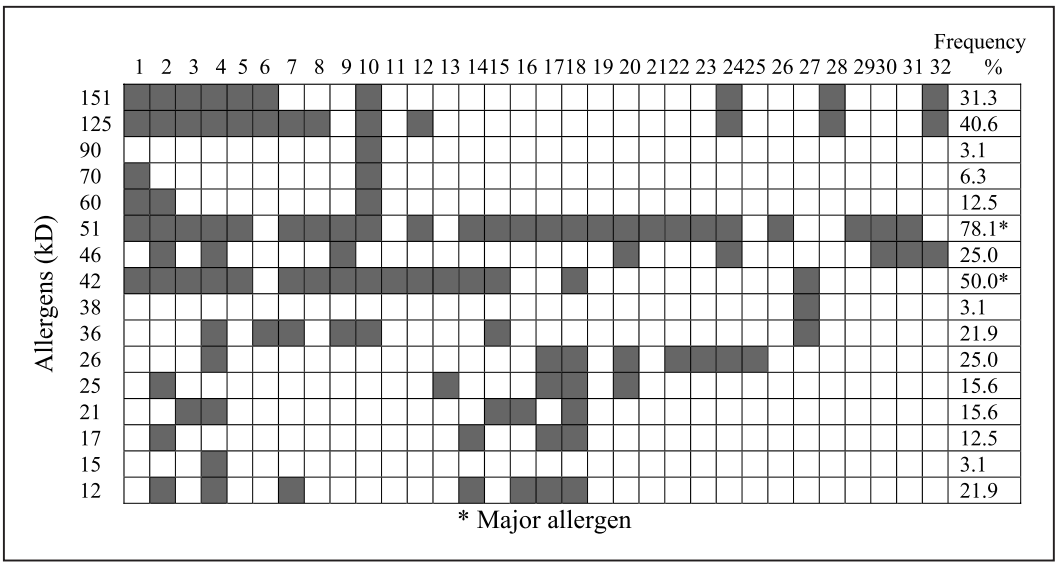
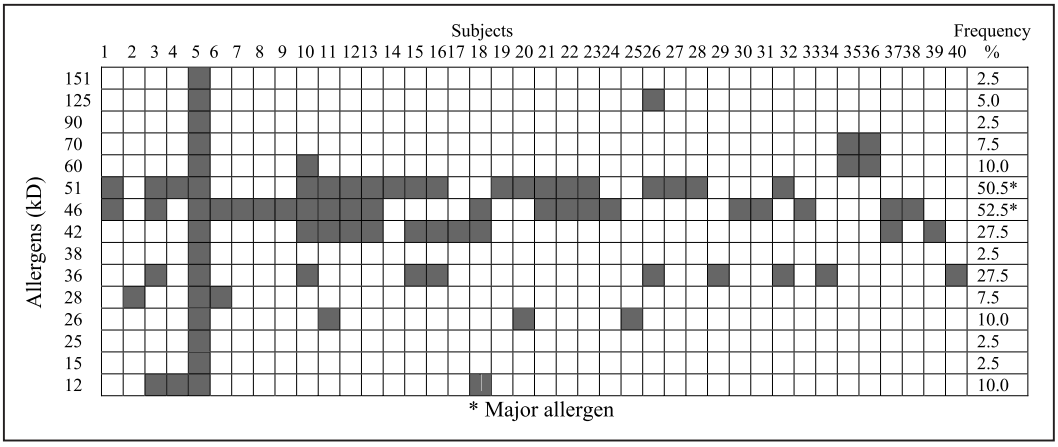


Table 2. The frequency of specific-IgE binding proteins in 32 patients sensitized to golden snapper



Most seafood allergens are stable molecules and resist the effect of cooking, processing or digestive process (Lopata & Potter, 2001). However, we found that the major allergens for both fish species were heat-labile allergens. Fish allergens between 40 and 90 kD have been reported to be heat-sensitive (Bernhisel-Broadbent *et al.*, 1992). Several heat-sensitive allergens in the range of 85 to 40 kD have been reported in sole and hake (Porcel *et al.*, 2001), and in the range of 37 to 50 kD in angler fish (Mugica *et al.*, 2003).

Several other immunoblotting studies have also reported IgE-binding proteins at 40 kD in tuna (James *et al.*, 1997), tropical sole (Asero *et al.*, 1999) and eel, eelpout and codfish (Sten *et al.*, 2004). We believe that the ~42 kD of heat-labile allergen found in our study might correspond to the

41 kD protein, homologous to an enzyme aldehyde phosphate dehydrogenase (APDH) as reported by Das Dores *et al.* (2002b). It should be noted that enzymes including APDH are mainly heat-sensitive and will be denatured in high temperature (Hendrickx *et al.*, 1998). Other studies have also reported potential allergens at ~50 and 46 kD, but are not well characterized (Yamada *et al.*, 1999; Das Dores *et al.*, 2002a). It is possible that our 46 kD might be similar to the 46 kD reported by Yamada *et al.* (1999), while the ~51 kD might be a parvalbumin tetramer as described by Das Dores *et al.* (2002a).

Parvalbumin, a ~12 kD heat and denaturation resistant protein has been widely reported as the major allergen and responsible in the majority of allergic reactions to various fish species (Bernhisel-Broadbent *et al.*, 1992; Hamada *et al.*, 2001; Porcel *et al.*, 2001). Although our immunoblotting results demonstrated specific IgE-binding to the ~12 kD protein corresponding to fish parvalbumin, it was seen only as minor allergens. This result implies that this protein may not play an important role in allergy to our local snapper.

In this study, minor allergens of high molecular weight (>100kD) were demonstrated for both fish species but was more prominent in red snapper. Protein profile of both showed that these allergens were resistant to heat. We believe that these proteins might be collagen, a new thermostable fish allergen with high molecular weight (~100 kD), which has also been identified as a highly cross-reactive fish allergen (Hamada *et al.*, 2003a).

In this study, the ~12 kD protein was not seen as major allergen, instead a ~51 kD protein was identified as the major allergen of both fish species. Proteins of 46 and 42 kD molecular weight were also demonstrated as major allergens of golden snapper and red snapper, respectively. All these major allergens were found to be heat-sensitive proteins.

Acknowledgements. The authors wish to thank the Director of Institute for Medical Research for permission to publish this paper.

REFERENCES

Asero, R., Mistrello, G., Roncarolo, D., Casarini, M. & Falagiani, P. (1999). True monosensitivity to a tropical sole. *Allergy* **54**(11): 1228-1229.

Bernhisel-Broadbent, J., Scanlon, S.M. & Sampson, H.A. (1992). Fish hypersensitivity: *In vitro* and oral challenge results in fish-allergic patients. *Journal Allergy Clinical Immunology* **89**: 730-707.

Bugajska-Schretter, A., Grote, M., Vangelista, L., Valent, P., Sperr, W.R., Rumpold, H., Pastore, A., Reichelt, R., Valenta, R. & Spitzauer, S. (2000). Purification, biochemical and immunological characterisation of a major food alergen: different immunoglobulin E recognition of the apo- and calcium-bound forms of carp parvalbumin. *Gut* **46**(5): 661-669.

Das Dores, S., Chopin, C., Romana, A., Galland-Irmouli, A.V., Quaratino, D., Pascual, J., Fleurence, J. & Gueant, J.L. (2002a). A new oligomeric parvalbumin allergen of Atlantic cod (Gad m1) encoded by a gene distinct from that of Gad c1. *Allergy* **57**(72): 84-74.

Das Dores, S., Chopin, C., Villaume, C., Fleurence, J. & Gueant, J.L. (2002b). IgE-binding and cross-reactivity of a new 41kDa allergen of codfish. *Allergy* **57**(72): 79-83.

Hamada, Y., Nagashima, Y. & Shiomi, K. (2001). Identification of collagen as a new fish allergen. *Bioscience Biotechnology & Biochemical* **65**(2): 285-291.

Hamada, Y., Nagashima, Y., Shiomi, K., Shimojo, N., Kohno, Y., Shibata, R., Nishima, S., Ohsuna, H. & Ikezawa, Z. (2003a). Reactivity of IgE in fish-allergenic patients to fish muscle collagen. *Allergology International* **52**: 139-147.

Hamada, Y., Tanaka, H., Ishizaki, S., Ishida, M., Nagashima, Y. & Shiomi, K. (2003b). Purification, reactivity with IgE and cDNA cloning of parvalbumin as the major allergen of mackerels. *Food & Chemical Toxicology* **41**: 1149-1156.

Helfe, S.L. (1996). The molecular biology of food allergy. *Immunology & Allergy Clinics of North America* **16**(3): 565-590.

Hansen, T.K., Bindslev-Jensen, C. Skov, P.S. & Poulsen, L.K. (1997). Codfish allergy in adults: IgE cross-reactivity among fish species. *Annals of Allergy Asthma & Immunology* **78**: 187-194.

Hendrickx, M., Ludikhuyze, L., Broeck, V. & Weemaes, C. (1998). Effect of high pressure on enzymes related to food quality. *Trends in food Science & Technology* **9**(5): 197-203.

James, J.M., Helm, R.M., Burks, A.W. & Lehrer, S.B. (1997). Comparison of pediatric and adult IgE antibody binding to fish protein. *Annals of Allergy Asthma Immunology* **79**: 131-137.

Jeebhay, M.F., Robbins, T.G., Lehrer, S.B. & Lopata, A.L. (2001). Occupational seafood allergy. *Occupational Environment Medicine* **58**: 553-562.

Lopata, A.L. & Potter, P.C. (2001). Allergy and other adverse reactions to seafood. *ACI International* **13**: 271-281.

Mohsin, A.K.M. & Ambak, M.A. (1996). *Marine fishes & fisheries of Malaysia and neighbouring countries*. Serdang: Universiti Putra Malaysia. 515-517.

Mugica, M.V., Anibarro, B., Seoane, F.J. & Lombardero, M. (2003). Contact urticaria by anger fish. *Allergy* **58**: 680-690.

Porcel, S., Leon, F., Cumplido, J., Cuevas, M., Guimaraens, D. & Conde-Salazar, L. (2001). Contact urticaria caused by heat-sensitive raw fish allergens. *Contact Dermatitis* **45**: 139-142.

Shahnaz, M., Gendeh, B.S. & Nasuruddin, A. (2001). Skin test reactivity to inhalant and food allergens in patients with allergic rhinitis. *International Medical Research Journal* **5**(2): 69-73.

Sten, E., Hansen, T.K., Skov, P.S., Andersen, S.B., Torp, A.M., Bindslev-Jensen, U., Bindslev-Jensen, C. & Poulsen, L.K. (2004). Cross-reactivity to eel, eelpout and ocean pout in codfish-allergic patients. *Allergy* **59**: 1173-1180.

Van Do, T., Hordvik, I., Endresen, C. & Elsayed, S. (1999). Expression and analysis of recombinant salmon parvalbumin, the major allergen in Atlantic Salmon (*Salmo salar*). *Scandinavian Journal of Immunology* **50**: 619-625.

Yamada, S., Nolte, H. & Zychlinsky, E. (1999). Identification and characterization of alergens in two species of tuna fish. *Annals of Allergy Asthma & Immunology* **82**: 395-400.