

## What we know about fish allergy by the end of the decade?

Running title: Advances in fish allergy in 2018.

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## Abstract

Fish allergy is one of the most common food allergies. It is usually considered to be immunoglobulin (Ig) E-mediated and correlated well with diagnostic tests such as prick tests and/or specific IgE. Recommended treatment is avoidance, generally extended to all fishes. However, new clinical presentations have been described, including non-IgE-mediated disease, mono-sensitization, and new syndromes that are sometimes associated with surprising cross-reactivity. Advances in molecular allergy have provided insights into new allergens and have increased our understanding of cross-reactivity. This paper focuses on recent publications providing information for clinicians involved in the management of fish allergy.

**Keywords:** food allergy, fish, clinical aspects, allergens, parvalbumin, pseudo allergy, cross-reactivity.

## Resumen

La alergia al pescado es una de las alergias alimentarias más frecuentes. Constituye habitualmente una alergia IgE mediada que se identifica correctamente mediante las pruebas cutáneas y/o la IgE específica in vitro. El tratamiento recomendado es la evitación de la ingesta habitualmente de todo tipo de pescados. Sin embargo, hay otras formas de presentación como la alergia no-IgE mediada, monosensibilizaciones y nuevos síndromes asociados a otros tipos de reactividad cruzada. Los avances en el diagnóstico molecular han descrito nuevos alérgenos y aumentado nuestro conocimiento sobre la reactividad cruzada. Este artículo analiza las publicaciones recientes que proporcionan nueva información para el tratamiento de la alergia a pescados.

**Palabras clave:** Alergia alimentaria. Pescado. Aspectos clínicos. Alérgenos. Parvalbúmina. Pseudoalergia. Reactividad cruzada.

## Introduction

Fish consumption has grown in recent years due to its nutritional content, and this increase has been associated with an increase in fish allergy. The prevalence of fish allergy is variable and depends on local availability and consumption patterns; indeed, its prevalence is difficult to estimate because of confusion with pseudo-allergic reactions and reactions to shellfish. However, the prevalence is generally considered to be lower than 1%. Fish allergy is essentially immunoglobulin E- (IgE-) mediated, but some non-IgE-mediated reactions have been described, especially in pediatric patients. Fish allergens can be transmitted by ingestion, by inhalation, or by skin contact, and the clinical manifestations can be mild, moderate, or severe. Cross-reactivity between various fish species is frequent, but patients who are mono-allergic to one fish type can tolerate exposure to other types without adverse reactions. Some surprising cross-reactions have been described between fish and other food. This article discusses the most recent findings on fish allergy.

## Epidemiology

Fish allergy is one of the eight main food allergies and can affect both children and adults [1,2]. Labelling of the industrialized countries requires its mandatory declaration regardless of the quantity or proportion in which it is part of the final product. The prevalence of fish allergy is not known, but less than 1% of the general population appear to have fish allergy, with a range of 0% to 8%, depending on the study population's food habits, the diagnostic criteria used in the study, the mode of exposure and the age of the population. It is more often seen in countries with higher fish consumption, such as Australia, Asia, and parts of Europe (Spain, Portugal, and Scandinavians countries) [2,3]. A 2016 review of 7333 articles identified by

Moonesinghe et al., of which 61 studies met the inclusion criteria and were included in this review, which reported that the prevalence of fish allergy varies from 0% to 7%, depending on the diagnostic method used i.e. self-questionnaire, prick test (PT), IgE-based test, clinical history, or sensitization. When food challenge tests were used, the prevalence was just 0.3% [3]. In Norway, 3% of food allergies at the age of 2 (n=3623) are attributed to fishes. In the United States, the prevalence of allergy to seafood was 5.9% in 14,948 study participants; 0.4% were allergic to fish, with the majority of these (67%) being allergic to multiple fish species [5]. In a recent study of 4400 adults in the United States, the population-based prevalence of fish allergy in response to a phone survey was approximately 0.7% [5]. In Asian countries, there is a higher prevalence of 2.29% in the Philippines versus 0.26% in Singapore and 0.29% in Thailand [4].

Occupational allergy due to fish allergen exposure was first described in 1937 by Besche [5]. The prevalence of occupational asthma due to fish allergens exposure is estimated to account for 2% to 8% of occupational asthma among exposed individuals [1,6] and occurs mainly in countries where the fishing industry is important.

### **Biological classification**

Fish species are divided into two main groups: cartilaginous fish (chondrichthyes: sharks and rays); and bony fish and osteichthyes, which includes two classes, sacropterygii (lobe-finned fish: lungfish, coelacanth) and actinopterygii (ray-finned fish: teleosts) [4,7,8]. Of the 30000 known species of fish, most are teleosts. A limited number of species are frequently consumed: Salmoniformes (salmons and trouts), Gadiformes (cods and hakes), Perciformes (mackerels and

tunas), Clupeiformes (herrings and sardines), Cypriniformes (carps and goldfish), Siluriformes (catfish), and Pleuronectiformes (sole, flounder, turbot, and halibut) [1,5,9,10].

### **Clinical aspects of fish allergy**

Fish allergy is generally IgE-mediated, and affected patients present with immediate clinical signs, which are usually severe. The classic clinical signs include oral allergy syndrome, cutaneous symptoms (diffuse urticaria, angioedema), gastrointestinal manifestations (abdominal pain, diarrhea, and sudden-onset vomiting), respiratory symptoms (rhinitis, asthma), and in the most severe cases, anaphylactic shock. Respiratory symptoms can occur due to vapor inhalation while cooking fish [6]. Non-IgE-mediated reactions have mainly been reported in pediatric cases as food protein-induced enterocolitis syndrome (FPIES). The clinical presentation is different from IgE-mediated symptoms. Acute FPIES manifests within 1-4 hours after ingestion with repetitive emesis, pallor, and lethargy progressing to dehydration and in some cases hypovolemic shock. Chronic FPIES manifests with intermittent emesis, watery diarrhea, and poor growth progressing to dehydration. Diagnosis of FPIES relies on recognition of a pattern of clinical symptoms [11,12,13]. Fish is one the most frequent FPIES trigger in Mediterranean countries [14]. Study from Vazquez-Otiz et al. recently conducted in Spain, showed that of 81 children who presented with FPIES fish was the main trigger in 54.3% of the cases [11]. In another European series, FPIES due to fish was also common but only represented 12% to 15% of FPIES cases [15,16,17]. Notably, these results differed from those found in other countries, particularly in the United States, where FPIES is frequently due to multiple foods [11]. FPIES induced by fish has some particularities compared to

those produced by other foods: it begins later (except those caused by shellfish) and has a later resolution, at least than those produced by milk. FPIES has also been described for fish in adults and adolescents, being in them the second cause after shellfish [18,19].

Occupational allergy due to exposure to fish allergens can lead to upper and lower (rhinitis, asthma) respiratory symptoms, conjunctivitis, contact dermatitis, and urticaria. Anaphylaxis due to cutaneous contact has also been reported [6,20]. Few clinical studies have investigated the minimal eliciting doses for fish allergy. However, very low amounts of fish (in the milligram range) seem to be sufficient for triggering allergic symptoms in sensitized patients and in the Europrevall study, the ED10 was established in 27 mg [21,22]. This could explain the allergic reactions in certain patients to traces of protein in fish oils.

Little is known about the natural history of fish allergy. For IgE-mediated allergies, sensitization generally starts during childhood and often persists until adulthood [23,24], although some patients develop a clinical tolerance [20]. Published studies indicate that the evolution is variable for FPIES. In a Spanish cohort, 75% of children with FPIES triggered by fish acquired tolerance by 5 years of age [11], while other studies in Europe reported tolerance in 19% to 36% of studied cases [15,16,17].

## **Allergens**

The identification and accurate characterization of fish allergens from distinct species and regions allow more precise diagnoses and facilitate the prevention of allergic reactions [26]. However, at the molecular level, less than 0.5% species have

been analyzed. The analyses have mainly included fish that are commonly consumed in Europe, such as carp, salmon, trout, tuna, and cod [5,10].

## Parvalbumin

Parvalbumin proteins represent the major fish allergen. These small (10- to 12-kDa) muscle proteins belong to the family of calcium-binding proteins and are resistant to enzymatic digestion and to heat [4]. Elsayed et al. identified the first fish allergen, parvalbumin from Baltic cod (Gad c 1 or allergen M), in 1969. Since then, parvalbumin has been identified as an allergen in other species as well, including in salmon (Sal s 1), mackerel (Sco a 1, Sco s 1 and Sco j 1), carp (Cyp c 1), and several species of tropical fish [4,23].

The parvalbumin protein is globular in shape and contains six helices, termed helices A–F. Parvalbumin is an EF-hand calcium-binding protein; these proteins are characterized by the presence of a helix, a loop, and a second helix, with the two helices arranged like the spread thumb and index finger of a human hand [1,4]. Calcium binds to these helices via ionic bonds, which results in conformational stability. Two parvalbumin isoforms have been identified, alpha and beta. Parvalbumin alpha is found mainly in cartilaginous fishes and does not seem to be allergenic. The clinical cross-reactivity between alpha- and beta-parvalbumin is very low in both fish classes (bony and cartilaginous), although this phenomenon is not fully understood. Most of the sequenced fish parvalbumin proteins are beta isoforms, which are found in bony fishes [1,4]. Teleost fish have two types of muscle, white muscle (also called light muscle) used for short bursts of swimming and dark muscle (also called red muscle) which is located directly under the skin, that is for continuous swimming. White muscle fibers with fast contraction have the highest concentration



of parvalbumin such as flounder and cod while red slow contracting muscle fibers such in as tuna and skipjack contain lower levels. Since the proportion of white and red muscle varies between species, the parvalbumin content also varies in different species [1,27]. Large migratory fish, such as those in the Xiphiidae family (e.g. swordfish), have lower parvalbumin content than small sedentary fish (e.g. cod, carp, redfish and herring) [28,29]. For example, swordfish have less than 1 mg of parvalbumin per gram of fresh fillet, similar to the concentration found in tuna [23,29] and ~~meanwhile~~, other fish species, such as cod (*Gadus morhua*) and carp (*Cyprinus carpio*), have more than 2.5 mg of parvalbumin per gram [29]. Consequently, patients who are allergic to the parvalbumin in fish can tolerate certain species of bony fish with low parvalbumin concentrations, such as tuna and swordfish [28,29]. Additionally, the concentration of parvalbumin varies not only in different species but also in different parts of the fish, it is higher in dorsal than ventral and rostral regions than caudal [30].

Between 70% and 95% of patients who are allergic to fish have specific IgE against parvalbumin; the percentage varies depending on the type of fish used in the test and the studied population. Parvalbumin proteins from different fish species have a high level of sequence identity (>70%) and show structural similarity, which explains the observed parvalbumin IgE-dependent cross-reactivity. It is important to note that these allergens contain other epitopes in more variable regions of the parvalbumin protein. Such epitopes represent specific antigenic determinants for certain species, and the IgE reactivity to these epitopes correlates with clinical monosensitization to a single species of fish [1,4,7,9]. Example is exclusive allergy to salmonids by monosensitization to its parvalbumin [31].

Interestingly, beta-parvalbumin proteins from bony fish have a relatively high level of sequence identity with the alpha-parvalbumin in amphibian muscle (63% to 76%), reptiles (56% to 69%), and birds (54% to 71%) [4]. This may explain the clinical cross-reactivity between these species. Table 1 shows allergens that have been sequenced from various fish species [4,10].

### Other allergens

Other fish allergens have also been described. Both enolases and aldolases are clinically relevant to fish allergy, but the allergenicity of these proteins is less well established than the allergenicity of parvalbumin proteins [1,10]. Enolase (50 kDa) and aldolase (40 kDa) are enzymes involved in glucose metabolism. IgE reactivity against enolases and aldolases from tuna, salmon, and cod is found in patients both with and without parvalbumin sensitization. These two enzymes are less stable than parvalbumin. Interspecies cross-reactivity for enolase and aldolase is limited and clearly lower than that between parvalbumins. In 2013, a study by Kuehn et al. estimated the prevalence of fish allergy due to enolase and aldolase to be 63% for enolase and 50% for aldolase [24]. There was recently a case report of an 8-year-old patient with anaphylaxis due to swordfish. Four proteins were identified as involved allergens: pyruvate kinase, enolase, aldolase, and triosephosphate isomerase. This was the first report to describe these allergens in swordfish; notably, parvalbumin was not involved in the reaction [29].

Collagen was identified as a fish allergen in the early 2000s. Collagen is a rod-shaped protein of about 330 kDa that is present mainly in skin. A Japanese study (n=36) in 2016 showed that 50% of patients who were allergic to fish had IgE reactivity to mackerel collagen [32]. Collagen from cartilaginous fish has lower

allergenicity than collagen from bony fish [33]. Gelatin (collagen type I) is often used in the food industry and in pharmaceuticals to replace mammalian gelatin. The allergenicity of gelatin differs from that of collagen because of the destruction of some epitopes by the hydrolysis process. Fish collagen and gelatin found respectively in dietary supplements and marshmallows have been involved in allergic reaction [35,36]. Gelatin poses an allergenic risk due to potential contamination by parvalbumin. Indeed, traces of parvalbumin have been detected in isinglass, a type of gelatin that is used in the clarification of wine and that has, until now, been considered non-allergenic [37].

Tropomyosin are alpha-helical proteins belonging to the family of actin binding proteins. There are numerous isoforms in molds and in both muscle and non-muscle cells of animals [38]. Tropomyosin is considered an invertebrate pan-allergen, and the tropomyosin found in vertebrates has not classically been considered as allergenic. Nevertheless, its allergenicity was first described in 2013 in tilapia from Mozambique (*Oreochromis mossambicus*) [34,39]. Subsequently, another group that used immunoblotting reported IgE-mediated immunoreactivity to tropomyosin from cod, yellow tuna, and swordfish in 10 of a cohort of 19 patients who presented with recurrent type I hypersensitivity following fish ingestion. It is particularly interesting to note that these patients had negative PTs for commercial fish extracts [40]. The homology between invertebrate tropomyosin and fish tropomyosin is only 57%, which theoretically is not sufficient to lead to clinical cross-reactivity [39]. However, Peixoto et al described a child suspected of clinical cross-reactivity between fish and shrimp tropomyosin [41].

Vitellogenin is a protein found in fish eggs. It is resistant to enzymatic digestion, suggesting that the relevant allergens are sub-fragments of vitellogenin,

such as lipovitellin and  $\beta'$ -component ( $\beta'$ -c). Clinical reactivity to vitellogenin is variable and is often specific to certain types of caviar, but cross-reactivity to other fish eggs has been reported, especially cross-reactivity between salmon eggs and herring eggs [42,43]. No cross-reactivity with chicken egg-equivalent proteins has been found [43,44].

Mention should be made of other potential allergens of fish of unknown relevance such as aldehyde phosphate dehydrogenase, triosephosphate isomerase, glyceraldehyde-3-phosphatide dehydrogenase and creatine kinase [45,46].

### **Cross-reactivity**

There is frequently clinical cross-reactivity between different fish species, which is why patients with fish allergy are often advised to avoid all species of fish and all fish-derived products [9]. However, numerous patients who are allergic to one fish species seem to tolerate other species. This difference in reactivity can be explained by the phylogenetic dispersion of species and by different allergen content [23]. For example, a patient who is allergic to the parvalbumin in fish may be able to tolerate certain species of fish that have low parvalbumin content, such as tuna and swordfish [28,29,47]. To date, monosensitivity has been described for sole, swordfish, pangasius/tilapia, tuna/marlin, cod and, more recently, for salmon [21,48,49].

Until recently, the weak clinical cross-reactivity between parvalbumin alpha and beta in the two classes of fish (cartilaginous and bony) has not been understood [1,4]. A study by Kalic et al. of 17 patients with fish allergy showed that the specific IgE level and basophil activation were significantly lower for alpha parvalbumin from shark and ray than for beta parvalbumin from the studied bony fishes. In some

patients, the absence of clinical reactivity to ray was confirmed by an oral challenge test. The researchers concluded that cartilaginous fish are well tolerated by patients allergic to bony fish, thereby helping the patients avoid useless food restriction [50]. In the same sense Calderon-Rodriguez et al [51] describe good tolerance to dogfish (a small shark frequently consumed in southern Spain) in patients allergic to bony fish. Also, the collagen of the cartilaginous fish seems to be less allergenic than that of the bony fish [33].

Cross-reactivity with other meat sources has also been described. For amphibians, the cross-reactivity between fish and frog involves beta parvalbumin [52]. Recently, Haroun-Diaz et al. reported the first case of anaphylaxis due to cross-reactivity between fish and crocodile meat [53]. Kuehn et al. reported clinical cross-reactivity to poultry in a cohort of 36 patients that showed allergies to both fish and chicken meat, termed fish-chicken syndrome. Notably, both enolase (Gal d 9) and aldolase (Gal d 10) in chicken meat play major roles in this syndrome, as does parvalbumin (Gal d 8) [1,7,34,54]. Fish-chicken syndrome seems to have a low prevalence. A retrospective study of the results of PTs (n=3232) that were conducted between 2012 and 2016 at Brugmann University Medical Center looked at the prevalence of the association between sensitization to chicken and allergy to fish and found an association in 14% of cases (unpublished data). That study did not demonstrate immunological cross-reactivity; thus, we suspect that real cross-reactivity has an even lower prevalence. The sensitization profiles differed between the group of patients with an allergy to fish and the group with an allergy to chicken. The clinical presentation of subjects with allergies to both is usually severe and seems to be evolutionary.

Cross-reactivity to fish and other seafood (shellfish, mollusks) has not been demonstrated. Although such cross-reactivity has been suggested, the results of

molecular analysis have not yet confirmed this hypothesis [7,21]. Tropomyosin would be the trigger allergen, and fish tropomyosin shows a particular IgE specificity. However, serum samples from patients with shellfish allergy do not recognize any of the tropomyosin samples from tested fishes *in vitro*. One proposed hypothesis is that vertebrate tropomyosin is digested into smaller fragments than invertebrate tropomyosin and that these smaller fragments no longer form the three-dimensional IgE epitopes that are found in invertebrate tropomyosin [40].

### **Effects of Processing and Digestion on Allergenicity**

Some industrial processes modify parvalbumin allergenicity. For example, the allergenicity of canned tuna is lower than the allergenicity of some fresh fish, which explains why some patients with fish allergy show tolerance to canned tuna [55]. Notably, there are no reported allergies to surimi, which contains the flesh of pasteurized fish. This is probably explained by the intensive processing the fish undergoes during manufacturing. Aldolases and enolase seem to be more sensitive to heat and food processing than parvalbumins [24].

Gastric acidity also affects allergenicity. A comparison of fish digested in a pH 3 environments to fish digested in a more acidic pH 2 environments (which is the normal gastric pH in humans) demonstrates that higher pH allows some allergenicity to be preserved. Consequently, anti-acid drugs can lead to incomplete allergen digestion [4,5].

### **Diagnosis**

Patient history and dietary survey remain important elements in the diagnosis of fish allergy. PTs that use commercial fish extracts or fresh fish plus allergen-specific IgE

tests are routinely used to diagnose fish allergy. A PT is frequently used as the first diagnostic test because it is a rapid and low-cost assay. Nevertheless, PTs have low specificity and have a positive predictive value (PPV) less than 50% [5]. In addition, the efficacy of PTs is limited by frequent cross-reactivity that is not clinically relevant [1,4].

Allergen-specific IgE testing is generally a good indicator of sensitization to fish but its ability to distinguish clinical reactivity from immunological cross-reactivity is limited and cut-off depends on the studied population and has been reviewed by Garcia et al [56]. For example, for cod allergy, a specific IgE level of 20 KU/l has been shown to predict clinical reactivity [4,56]. While, other authors have described much lower cut-off points with high PPV (i.e. 0.35kU/L with PPV 91% in adults, 1.8kU/L with PPV 71% in children and adolescents) [56]. More than 30 fish extracts and two parvalbumin molecular allergens are available for use in specific IgE testing [1]. Notably, a negative result in specific IgE testing does not entirely exclude an allergic mechanism [57]. Gastrointestinal symptoms may not be accompanied by a positive PT or specific IgE test results [58]. Clinical reactivity can be verified by oral challenge, ideally double-blind, which is the gold standard for diagnosis [5].

### **Differential diagnosis**

*Anisakis* infestation (*Anisakis simplex*, helminth) results in the contamination of fish flesh by this parasite, which can cause severe allergic reactions in those who consume the fish. The symptoms can be digestive, cutaneous (urticaria, dermatitis), respiratory (asthma), and even anaphylactic. A new clinical concept of *Anisakis* allergy attributes this reaction not only to the presence of certain proteins derived from the parasite but also to the presence of viable parasites [59]. This hypothesis

was confirmed by an oral challenge test that used non-viable parasites, which was negative. Freezing at  $-20^{\circ}\text{C}$  for at least 24h is sufficient to kill the parasites [59]. Few studies have investigated the prevalence of *Anisakis* allergy. The current diagnostic approach is based on *in vitro* specific anti-*Anisakis* IgE testing and/or *in vivo* PT (when available) [5,60]. In a recent study in Italy, specific anti-*Anisakis* IgE (CAP) was not detected in 9 cases among 20 children with suggestive clinical history and positive PT [61].

Histamine poisoning or scombroid syndrome represents another cause of pseudo-allergy and stems from the high content of histamine in certain fish that have been badly preserved. Histamine is an endogenous amine that is produced by the conversion of histidine by histidine decarboxylase. The latter is found in bacteria that contaminate some preserved fish. The efficiency of histidine decarboxylase depends on temperature, on pH, and on the sodium concentration. Ideally, fish should be kept at a temperature of  $0^{\circ}\text{C}$  or lower so that the bacteria cannot proliferate and so that the histidine decarboxylase cannot be activated [62]. Scombroid syndrome is a benign illness that begins 10 to 30 min after ingestion of fish, and it spontaneously resolves within 24 h. The clinical presentation can be confused with that of fish allergy, but certain elements should prompt suspicion of a scombroid syndrome diagnosis, including abdominal pain, diarrhea, nausea and vomiting; facial or generalized erythema; urticaria and/or edema; headache or dizziness; xerostomia and metallic or bitter taste; and palpitations. Respiratory symptoms and low blood pressure are rare [63].



## Treatment

At present, the only treatment for fish allergy is strict avoidance of triggers and the use of adrenalin injection in patients with anaphylaxis. There are no published studies regarding specific oral immunotherapy, but the reduced allergenicity of canned tuna has allowed its use in the induction of tolerance. A 2011 study by Turner et al. of 167 patients with allergies to salmon and tuna showed that 20% of the patients can tolerate these two species if the fish is canned. The patients showed reduced papule size in a PT to ~~other species when they were exposed to allergenic extract from~~ salmon and tuna [55].

Some researchers have used subcutaneous desensitization with fish extracts, but this treatment remains experimental. Therapies that employ subcutaneous immunotherapy using hypoallergenic parvalbumin are currently being tested [64,65,66].

## Conclusion

Allergic reactions to fish can be immediate and severe. The diagnosis is based on clinical history, prick tests, specific IgE tests, and if needed, oral challenge tests. The main fish allergens seem to be parvalbumin, enolase, aldolase, and collagen. Optimal management requires careful reflection to avoid unnecessary restriction, including consideration of the patient's allergic profile at the molecular level. However, the number of available fish allergens is very limited. Therapeutic hypoallergenic parvalbumin is being developed, but randomized controlled studies in large series are still needed.

## References:

1. Kuehn A, Radauer C, Lopata A, Kleine-Tebbel J, Swoboda I. Extract-based and molecular diagnostics in fish allergy. *Molecular Allergy Diagnostics*. 2017;381-97.
2. Nwaru B.I, Hickstein L, Panesar S, Roberts G, Muraro A, Sheikh A. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy*. 2014;69:992-1007.
3. Moonesinghe H, Mackenzie H, Venter C, Kilbrun S, Turner P, Weir K. Prevalence of fish and shellfish allergy: A systematic review. *Ann Allergy Asthma Immunol*. 2016;117:264-72.
4. Stephen JN, Sharp MF, Reuthers T, Taki A, Campbell DE, Lopata AL. Allergenicity of bony and cartilaginous fish - molecular and immunological properties. *Clin Exp Allergy*. 2017;47: 300-12.
5. Sharp M, Lopata A. Fish Allergy: In Review. *Clinic Rev Allerg Immunol*. 2014;46:258–71.
6. Lopata A, Jeebhay M. Airborne Seafood Allergens as a Cause of Occupational Allergy and Asthma. *Curr Allergy Asthma Rep*. 2013;13:288–97.
7. Kuehn A, Codreanu-Morel F, Lehnert-Weber C, Doyen V, Gomez-André S-A, Bienvenu F. Cross-reactivity to fish and chicken meat – a new clinical syndrome. *Allergy*. 2016;71:1772-81.
8. Tsabouri S, Triga M, Makris M, Kalogeromitros D, Church M, Priftis Kostas P. Fish and Shellfish Allergy in Children: Review of a persistent Food Allergy. *Pediatric Allergy and Immunology*. 2012;23:608-15.
9. Kuehn A, Morisset M, Hilger. Fish Allergens beyond beta- Parvalbumin. *Revue Francaise d'Allergologie*. 2016;56:260-1.
10. Paulsen L, Morisset M, Kuehn A. EAACI Molecular Allergology User's Guide.
11. Vazquez-Ortiz M, Machinena A, Dominguez O, Alvaro M, Calvo-Campoverde K, Giner M et al. Food protein induced enterocolitis syndrome to fish and egg usually resolves by age 5 years in Spanish children. *J Allergy Clin Immunol*. 2017;5:512-5
12. Nowak-Węgrzyn A, Jarocka-Cyrta E, Moschione Castro A. Food Protein-Induced Enterocolitis Syndrome. *J Investig Allergol Clin Immunol*. 2017;27(1):1-18.
13. Vila Sexto L. Latest Insights on Food Protein-Induced Enterocolitis Syndrome: An Emerging Medical Condition. *J Investig Allergol Clin Immunol*. 2018;28:13-23.
14. Infante S., Marco-Martin G., Sanchez-Dominguez M., Rodriguez-Fernandez A., Fuentes-Aparicio V. et al. Food protein-induced enterocolitis syndrome by fish: Not necessarily a restricted diet. *Allergy*. 2018;73:728–732.
15. Vila L, García V, Rial MJ, Novoa E, Cacharron T. Fish is a major trigger of solid food protein-induced enterocolitis syndrome in Spanish children. *J Allergy Clin Immunol Pract* 2015;3:621-3.

16. Sopo SM, Monaco S, Badina L, Barni S, Longo G, Novembre E, et al. Food protein-induced enterocolitis syndrome caused by fish and/or shellfish in Italy. *Pediatr Allergy Immunol* 2015; 26:731-6.
17. González-Delgado P, Caparrós E, Moreno MV, Clemente F, Flores E, Velásquez L, et al. Clinical and immunological characteristics of a pediatric population with food protein-induced enterocolitis syndrome (FPIES) to fish. *Pediatr Allergy Immunol* 2016;27:269-75.
18. Tan JA, Smith WB. Non-IgE-mediated gastrointestinal food hypersensitivity syndrome in adults. *J Allergy Clin Immunol Pract*. 2014;2:355-7
19. Gonzalez-Delgado P, Caparrós E, Moreno MV, Cueva B, Fernández J. Food protein-induced enterocolitis-like syndrome in a population of adolescents and adults caused by seafood. *J Allergy Clin Immunol Pract*. 2018 Aug 1. pii: S2213-2198(18)30464-1. doi: 10.1016/j.jaip.2018.07.028.
20. Jeebhay MF, Lopata A L. Occupational allergy in seafood processing workers. *Adv Food Nutr Res* 2012;66:47-73.
21. Kuehn A, Swoboda I, Arumugam K, Hilger C, Hentges F. Fish allergens at a glance: variable allergenicity of parvalbumins, the major fish allergens. *Front Immunol*. *Frontiers in Immunology* 2014;5:179.
22. Ballmer-Weber BK, Fernandez-Rivas M, Beyer K, Defernez M, Sperrin M, Mackie AR et al. How much is too much? Threshold dose distributions for 5 food allergens. *J Allergy Clin Immunol*. 2015 Apr;135(4):964-71.
23. Schulkes K, Klemans R, Knigge L, De Bruin-Weller M, Bruijnzeel-Koomen C, Marknell deWitt A. Specific IgE to fish extracts does not predict allergy to specific species within an adult fish allergic population. *Clin Transl Allergy* 2014;4:27.
24. Kuehn A, Hilger C, Lehnert-Weber C, Coderanu-Morel F, Morisset M, Metz-Favre C et al. Identification of enolases and aldolases as important fish allergens in cod, salmon and tuna: component resolved diagnosis using parvalbumin and the new allergens. *Clin Exp Allergy* 2013;43:811-822.
25. Thalayasingam M, Lee BW. Fish and Shellfish Allergy. *Chem Immunol Allergy* 2015;101:152–161.
26. Rheuters T, Raith M, Sharp MF, Koeberl M, Stephen J, Nugraha R et al. Characterisation of Ras k 1 a novel major allergen in Indian mackerel and identification of parvalbumin as the major fish allergen in 33 Asia-Pacific fish species. *Clin Exp Allergy*. 2018 Apr;48:452-463.
27. Kobayashi A, Tanaka H, Hamada Y, Ishizaki S, Nagashima Y, Shiomi K. Comparison of allergenicity and allergens between fish white and dark muscles. *Allergy*. 2006;61:357-63.

28. Kobayashi A, Kobayashi Y, Shiomi K. Fish allergy in patients with parvalbumin-specific Immunoglobulin E depends on parvalbumin content rather than molecular differences in the protein among fish species. *Bioscience, Biotechnology and Biochemistry* 2016;80:2018-21
29. Valverde-Monge M, Pastor-Vargas C, Rodríguez del Rio P, Escudero C, Sánchez-García S, Mendez Brea P et al. Anaphylaxis by exclusive allergy to swordfish and identification of a new fish allergen. *Pediatr Allergy Immunol.* 2018;29:563-5.
30. Kubota H, Kobayashi A, Kobayashi Y, Shiomi K, Hamada-Sato N. Reduction in IgE reactivity of Pacific mackerel parvalbumin by heat treatment. *Food Chem.* 2016;206:78-84.
31. Kuehn A, Hutt-Kempf E, Hilger C, Hentges F. Clinical monosensitivity to salmonid fish linked to specific IgE-epitopes on salmon and trout beta-parvalbumins. *Allergy.* 2011;66:299-301.
32. Kobayashi Y, Akiyama H, Hüge J, Kubota H, Chikazawa S, Satoh T. Fish Collagen is an important panallergen in the Japanese population. *Allergy* 2016;71:720-723.
33. Kobayashi Y, Kuriyama T, Nakagawara R, Aihara M, Hamada-Sato N. Allergy to fish collagen: Thermostability of collagen and IgE reactivity of patients' sera with extracts of 11 species of bony and cartilaginous fish. *Allergol Int* 2016;65:450-458.
34. Hilger C, Van Hage M, Kuehn A. Diagnosis of allergy to mammals and fish: cross reactive vs specific markers. *Curr Allergy Asthma Resp* 2017;17:64 -76.
35. Kuehn A, Hilger C, Hentges F. Anaphylaxis provoked by ingestion of marshmallows containing fish gelatin. *J Allergy Clin Immunol.* 2009;123:708-709.
36. Fujimoto W, Fukuda M, Yokooji T, Yamamoto T, Tanaka A, Matsuo H. Anaphylaxis provoked by ingestion of hydrolysed fish collagen probably induced by epicutaneous sensitization. *Allergol Int.* 2016;65:474-476.
3729. Walker S, Donet Camarena C, Freeman G. Alternatives to Isinglass for Beer Clarification. *J. Inst. Brew* 2007;113:347-354.
38. Geeves M, Hitchcock-DeGregori S, Gunning P. A systematic nomenclature for mammalian tropomyosin isoforms. *J Muscl Res Cell Mtil.* 2015;36:147-153.
39. Liu R, Holck L, Yang E, Liu C, Xue W. Tropomyosin from tilapia (*Oreochromis mossambicus*) as an allergen. *Clin Exp Allergy.* 2013;43:365-377.
40. González-Fernández J, Alguacil-Guillén M, Cuéllar C, Daschner A. Possible Allergenic Role of Tropomyosin in Patients with Adverse Reactions after Fish Intake. *Immunol Invest.* 2018;47:416-29.
41. Peixoto S, Monteiro T, Carvalho M, Santos M, Matos C, Bartolomé B, Labrador-Horrillo M, Quaresma M. Vertebrate Tropomyosin as an Allergen. *J Investig Allergol Clin Immunol.* 2018;28:51-3.

42. González-De-Olano D, Rodríguez-Marco A, González-Mancebo E, Gandolfo-Cano M, Meléndez-Baltanás A, Bartolomé B. Allergy to red caviar. *J Investig Allergol Clin Immunol* 2011;21:493-4.
43. Kondo Y, Kakami M, Koyama H, Yasuda T, Nikajima Y, Kawamura M et al. IgE cross-reactivity between fish (Salmon, Herring and Pollock) and chicken egg in patients anaphylactic to salmon roe. *Allergol Int.* 2005;54:317-23.
44. Perez-Gordo M, Sanchez-Garcia S, Cases B, Pastor C, Vivanco F, Cuesta-Herranz J. Identification of vitellogenin as an allergen in Beluga caviar allergy. *Allergy.* 2008;63:479-480.
45. Larco-Rojas X, González-Gutiérrez ML, Vázquez-Cortés S, Bartolomé B, Pastor-Vargas C, Fernández-Rivas M. Occupational Asthma and Urticaria in a Fishmonger Due to Creatine Kinase, a Cross-Reactive Fish Allergen. *J Investig Allergol Clin Immunol.* 2017;27:386-8.
46. Ruethers T, Taki AC, Johnston EB, Nugraha R, Le TTK, Kalic T, McLean TR, Kamath SD, Lopata AL. Seafood allergy: A comprehensive review of fish and shellfish allergens. *Mol Immunol.* 2018 Aug;100:28-57.
47. Sørensen M, Kuehn A, Mills C, Costello C, Ollert M, Småbrekke L et al. Cross-reactivity in Fish Allergy: A Double-Blind Placebo-Controlled Food Challenge Trial. *J Allergy Clin Immunol.* 2017;140:1170-1172.
48. Viñas M, Pineda F, Izquierdo-Domínguez A, Castillo M, Castillo MJ, Hernández N, Delavalle B, Barrena J, Ibero M. Allergy To Limanda aspera (Yellowfin Sole): Report of a Case of Food Allergy in a Child. *J Investig Allergol Clin Immunol.* 2018;28:137-8.
49. Ebo DG, Kuehn A, Bridts CH, Hilger C, Hentges F, Stevens WJ. Monosensitivity to pangasius and tilapia caused by allergens other than parvalbumin. *J Investig Allergol Clin Immunol.* 2010;20(1):84-8.
50. Kalic T, Morel-Codreanu F, Radauer C, Ruethers T, Taki AC, Swoboda I et al. Patients Allergic to Fish Tolerate Ray Based on the Low Allergenicity of Its Parvalbumin. *J Allergy Clin Immunol Pract.* 2018 Nov 22. pii: S2213-2198(18)30738-4. doi:10.1016/j.jaip.2018.11.011.
51. Calderon-Rodriguez S, Pineda F, Perez R, Muñoz C. Tolerability to dogfish in children with fish allergy. *Allergol Immunopathol (Madr).* 2016 Mar-Apr;44(2):167-9.
52. Hilger C, Thill L, Grigioni F, Lehnert C, Falagiani P, Ferrara A et al. IgE antibodies of fish allergic patients cross-react with frog parvalbumin. *Allergy* 2004;59:633-660.
53. Haroun-Díaz E, Blanca-López N, Vázquez de la Torre M, Javier Ruano, Somoza Álvarez ML, Labrador Horrillo M et al. Severe anaphylaxis due to crocodile meat allergy exhibiting wide crossreactivity with fish allergens. *J Allergy Clin Immunol Pract.* 2018;6:669-70.

54. Kuehn A, Fisher J, Hilger C, Sparla C, Biedermann T, Hentges F. Correlation of clinical mono-sensitivity to cod with specific IgE to enolase and aldolase. *Ann Allergy Asthma Immunol* 2014;113:670-671.
55. Turner P, Ng I, Kemp A, Campell D. Sea -food allergy in children: a descriptive study. *Ann Allergy Asthma Immunol* 2011;106:494-501.
56. García BE, Gamboa PM, Asturias JA, López-Hoyos M, Sanz ML, Caballero MT et al. Guidelines on the clinical usefulness of determination of specific immunoglobulin E to foods. *J Investig Allergol Clin Immunol*. 2009;19(6):423-32.
57. Barbarroja-Escudero et al. Bleak allergy: First case report. *Allergology International* 2016; 65;472-473
58. Chikazawa S, Hashimoto T, Kobayashi Y, Satoh T. Fish collagen allergy: a pitfall of the prick-to-prick test with raw fish. *Br J Dermatol* 2015;173:1330–31.
59. Daschner A, Cue´llar C, Rodero M. The Anisakis allergy debate: does an evolutionary approach help? *Trends Parasitol*. 2012;28;9-15.
60. Nieuwenhuizen NE, Lopata AL. Allergic reactions to Anisakis found in fish. *Curr Allergy Asthma Rep*. 2014;14:455
61. Tripodi S, Pingitore G, Calvani M, Scala G, Rodriguez-Perez R, Sfika I, Asero R. Anisakis Sensitivity in Italian Children: A Prospective Study. *J Investig Allergol Clin Immunol*. 2017;27:142-3.
62. Feng C, Teuber S, Gershwin ME. Histamine (Scombroid) Fish Poisoning: a Comprehensive Review. *Clin Rev Allergy Immunol*. 2016;50:64-9.
63. Ridolo E, Martgnago I, Senna G, Ricci G. Scombroid syndrome: it seems to be fish allergy but... it isn't. *Curr Opin Allergy Clin Immunol* 2016;16:516-21.
64. Zuidmeer-Jongejan L, Huber H, Swoboda I, Rigby N, Versteeg SA, Jensen BM et al. Development of a Hypoallergenic Recombinant Parvalbumin for First-in-Man Subcutaneous Immunotherapy of Fish Allergy. *Int Arch Allergy Immunol*. 2015;166:41-51.
65. Buonomo A, Nucera E, Schiavino D. Doctor, can I desensitize my food-allergic child using directly the allergenic molecules? *Curr Opin Allergy Clin Immunol*. 2016;16:278-83.
66. Doularidis N, Linhart B, Swoboda I, Gstotner A, Vassilopoulou E, Stolz F. In vivo allergenic activity of a hypoallergenic mutant of the major fish allergen Cyp c 1 evaluated by means of skin testing. *J Allergy Clin Immunol*. 2016;136:493-5.

Table 1. Sequenced fish allergens

The list of fish allergens that have been sequenced with their family protein, their species and the order they belong to.

Order	species	Allergens	Protein
<b>Clupeiformes</b>	Herring ( <i>Clupea Harengus</i> )	Clu h 1	Beta-Parvalbumin
	Sardine ( <i>Sardinops sagax</i> )	Sar sa 1	Beta-Parvalbumin
<b>Cypriniformes</b>	Common carp ( <i>Cyprinus carpio</i> )	Cyp c 1	Beta-Parvalbumin
<b>Gadiformes</b>	Baltic cod ( <i>Morus callarias</i> )	Gad c 1	Beta-Parvalbumin
		Gad m 1	Beta-Parvalbumin
	Atlantic cod ( <i>Gadus morhua</i> )	Gad m 2	Enolase
		Gad m 3	Aldolase
<b>Perciformes</b>	Tuna ( <i>Thunnus albacares</i> )	Thu a 1	Beta-Parvalbumin
		Thu a 2	Enolase
		Thu a 3	Aldolase
	Indian mackerel ( <i>Rastrelliger kanagurta</i> )*	Ras K 1	Beta-Parvalbumin
<b>Pleuronectiformes</b>	Cardine ( <i>Lepidorhombus whiffiagonis</i> )	Lep w 1	Beta-Parvalbumin
<b>Salmoniformes</b>	Pacific Salmon ( <i>Oncorhynchus keta</i> )	Onc k 5	Vitellogenin
	Trout ( <i>Oncorhynchus mykiss</i> )	Onc m 1	Beta-Parvalbumin
	Atlantic Salmon ( <i>Salmo salar</i> )	Sal s 1	Beta-Parvalbumin
		Sal s 2	Enolase
		Sal s 3	Aldolase
<b>Scorpaeniformes</b>	Redfish ( <i>Sebastes marinus</i> )	Seb m 1	Beta-Parvalbumin