



Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Seafood allergy: A comprehensive review of fish and shellfish allergens

Thimo Ruethers^{a,b,c,d,1}, Aya C. Taki^{a,b,c,d,1}, Elecia B. Johnston^{a,c,d}, Roni Nugraha^{a,c,d,e},
 Thu T.K. Le^{a,c,d}, Tanja Kalic^f, Thomas R. McLean^g, Sandip D. Kamath^{a,b,c,d},
 Andreas L. Lopata^{a,b,c,d,*}

^a Molecular Allergy Research Laboratory, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia

^b Centre for Food and Allergy Research, Murdoch Childrens Research Institute, Melbourne, Australia

^c Centre for Biodiscovery and Molecular Development of Therapeutics, Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Australia

^d Centre for Sustainable Tropical Fisheries and Aquaculture, Faculty of Science and Engineering, James Cook University, Townsville, Australia

^e Department of Aquatic Product Technology, Bogor Agricultural University, Bogor, Indonesia

^f Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

^g Biotechnology Laboratory, School of Science, RMIT University, Bundoora, Victoria, Australia

ARTICLE INFO

Keywords:

Fish allergy
 Crustacean allergy
 Mollusc allergy
 IgE antibody
 Parvalbumin
 Tropomyosin

ABSTRACT

Seafood refers to several distinct groups of edible aquatic animals including fish, crustacean, and mollusc. The two invertebrate groups of crustacean and mollusc are, for culinary reasons, often combined as shellfish but belong to two very different phyla. The evolutionary and taxonomic diversity of the various consumed seafood species poses a challenge in the identification and characterisation of the major and minor allergens critical for reliable diagnostics and therapeutic treatments. Many allergenic proteins are very different between these groups; however, some pan-allergens, including parvalbumin, tropomyosin and arginine kinase, seem to induce immunological and clinical cross-reactivity.

This extensive review details the advances in the bio-molecular characterisation of 20 allergenic proteins within the three distinct seafood groups; fish, crustacean and molluscs. Furthermore, the structural and biochemical properties of the major allergens are described to highlight the immunological and subsequent clinical cross-reactivities. A comprehensive list of purified and recombinant allergens is provided, and the applications of component-resolved diagnostics and current therapeutic developments are discussed.

1. Classification of seafood

Seafood is comprised of the distinct taxa of fish (bony and cartilaginous fish), in the phylum of Chordata, and shellfish (crustacean and mollusc), in the phyla of Arthropoda and Mollusca, in the kingdom Animalia (Fig. 1). Aquatic animals of other phyla have not been investigated comprehensively regarding allergy due to limited consumption and hence are not discussed in this review. Other seafood, such as jellyfish (phylum Cnidaria), can trigger anaphylaxis upon ingestion (Imamura et al., 2013), but was reported to be safe for patients with allergy to the seafood groups mentioned above (Amaral et al., 2018).

1.1. Types of fish

Fish are divided into the superclasses of bony fish (Osteichthyes) and cartilaginous fish (Chondrichthyes). The third superclass of Vertebrata, the

tetrapods (Tetrapoda), contains all the four-limbed vertebrates including amphibians, birds, mammals, and reptiles. The bony fish are the largest group of all vertebrates consisting of 45 orders and over 435 families. They can be further divided into the two subclasses ray-finned fish (Actinopterygii) and lobe-finned fish (Sarcopterygii). Actinopterygii is the largest subclass and includes all edible bony fish. Most Sarcopterygii species are extinct, with the coelacanth being the oldest discovered order - having a continuous presence for 100 million years. Bony fish have an endoskeleton made of stable cranial bones, reproduce mostly externally, and are found in fresh and marine water whereas, cartilaginous fish have an endoskeleton made of cartilage, reproduce internally, and are found primarily in marine water. They can be further divided into the subclasses; Elasmobranchii and Holocephali. Elasmobranchii consist of sharks (Selachii), rays, skates (Batoidea), and sawfish. In contrast, the subclass Holocephali (ghost sharks) has only one surviving order (Chimaeriformes), which consists of rat fish, rabbit fish, and elephant fish. The most

* Corresponding author at: Pharmacy & Medical Research, Bldg 47, 1 James Cook Drive, James Cook University, Townsville, QLD, 4811, Australia.

E-mail address: andreas.lopat@jcu.edu.au (A.L. Lopata).

¹ TR and AT are equally contributing authors.

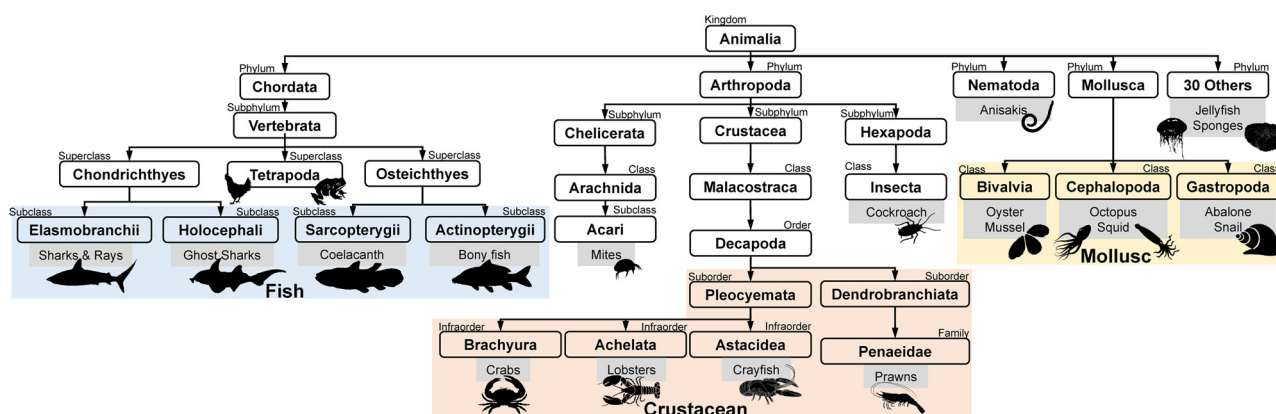


Fig. 1. Taxonomic tree of edible seafood species and related allergen sources. Classification and taxonomy of species are presented according to the NCBI (www.ncbi.nlm.nih.gov/taxonomy) and the Catalogue of Life (www.catalogueoflife.org/col/). Species in branches highlighted in blue, yellow, and red are considered as fish, mollusc, and crustacean, respectively. Allergic reactions to these and related species are discussed in this review (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

investigated species of ghost shark is the chimeric elephant fish, also known as the Australian Ghost Shark (*Callorhynchus milli*), as it is closely related to sharks, but also has distinct features like bony fish, such as gill covers. For consumption, and therefore allergy research, bony fish are the most relevant. Only in a few studies has the allergenicity of cartilaginous fish been investigated, and these suggest lower allergenicity compared to bony fish (Stephen et al., 2017).

1.2. Types of crustacean

Edible arthropods belong to the subphylum Crustacea and more specifically to the order Decapoda. This group includes prawns, shrimps, crabs, and lobsters (Fig. 1). Most crustaceans have five pairs of legs on the main thoracic body and five pairs of swimmerets on the abdomen or tail (Lee et al., 2012a). Crustaceans are closely related to arachnids (dust mites, spiders etc.) and insects such as cockroaches. Among the edible crustaceans, prawns constitute a major proportion of the consumed and farmed species. Decapods from the Penaeidae family are known as prawns, and those from the Caridae family, shrimps. Prawns and shrimps belong to two different taxonomical classifications with the main anatomical differences being the different overlapping pattern of the segments in the carapace and their brooding methods (Poore, 2004). Penaeids have a larger size compared to Caridae and are commercially important. Most of the species characterised for the existence and prevalence of allergens belong to this family. Shrimps are smaller in size and used mainly as additives or flavouring agents. Common examples of the caridean shrimps are the freshwater shrimp (*Macrobrachium rosenbergii*), and northern shrimp (*Crangon crangon*). However, the term “prawns” and “shrimps” are often used interchangeably in the commercial sector as well as in many research publications. The term prawn is used in the UK, Australia and other Commonwealth countries whereas the term shrimp is most commonly used in the USA and Europe. In addition, krill (*Euphausia superba*) are marine crustaceans that belong to the Euphausiidae family and are widely used in processed food products and the production of krill oil.

1.3. Types of molluscs

The mollusca are the second largest phylum of the kingdom Animalia with 104 edible species recorded by the Food and Agriculture Organization of the United Nations (FAO). This phylum consists of 9–10 taxonomic classes, with only three, Cephalopoda, Bivalvia, and Gastropoda, being commonly consumed by humans. In 2011 molluscs contributed around 12% of global fishery production; nearly 75% were farmed (Campbell and Pauly, 2013). Bivalvia is the most important

class commercially, with oysters, mussels, scallops, clams, and cockles constituting a significant part of total production. Bivalves have a shell consisting of two hinged parts, while cephalopod either have an internal calcareous cuttlebone (cuttlefish), an internal chitinous pen (squid), or no shell at all (octopus). About 80% of all mollusc species belong to the class of Gastropoda including abalone, snails, and slugs with very diverse anatomic features. Molluscs mainly inhabit aquatic environments. The only edible terrestrial molluscs are the land snails of the genus *Helix*.

2. Adverse reactions to seafood including IgE-mediated allergy

The clinical presentation of seafood allergy is similar to other food allergies. Single or multiple symptoms usually appear immediately or within two hours following exposure; however, late phase reactions of up to eight hours after ingestion have been reported (Lopata et al., 1997). Respiratory reactions along with oral allergy syndrome are reported more frequently than in other food allergies, and seafoods are one of the most common triggers of life-threatening anaphylactic reactions (Matricardi et al., 2016). Sensitisation and subsequent reactions occur most frequently upon ingestion; however, they can also occur due to skin contact or inhalation of aerosolised proteins generated during cooking or processing. Unlike most other food allergies, seafood allergy persists for life in up to 90% of patients, a trend also observed with peanut allergy.

Adverse reactions to seafood can be classified into three categories, based on underlying mechanisms: 1) Immunological reactions, including allergic reaction and Food Protein-Induced Enterocolitis Syndrome (FPIES), 2) Toxic reactions, including marine biotoxins and 3) Food intolerance. Adverse reactions due to toxins and/or food intolerance often resemble clinical symptoms of seafood allergy. A good patient workup and sensitive diagnostic analysis of IgE antibody reactivity can distinguish between a true seafood allergy and other adverse reactions.

This review focuses on IgE-mediated allergic reactions to seafood and the allergenic proteins involved. In contrast, adverse clinical reactions to seafood often involve non-immunological toxic reactions as detailed below (Fig. 2).

2.1. Adverse reactions to ingested seafood

In the USA, seafood is responsible for at least 1 in 6 food poisoning outbreaks and the proportion is even higher in Japan (Trevino, 1998). Diagnosis and treatment are generally similar to those for any other food poisoning. Seafood toxins are very stable, and their toxicity is not reduced by preparation procedures (e.g. cooking, pickling).

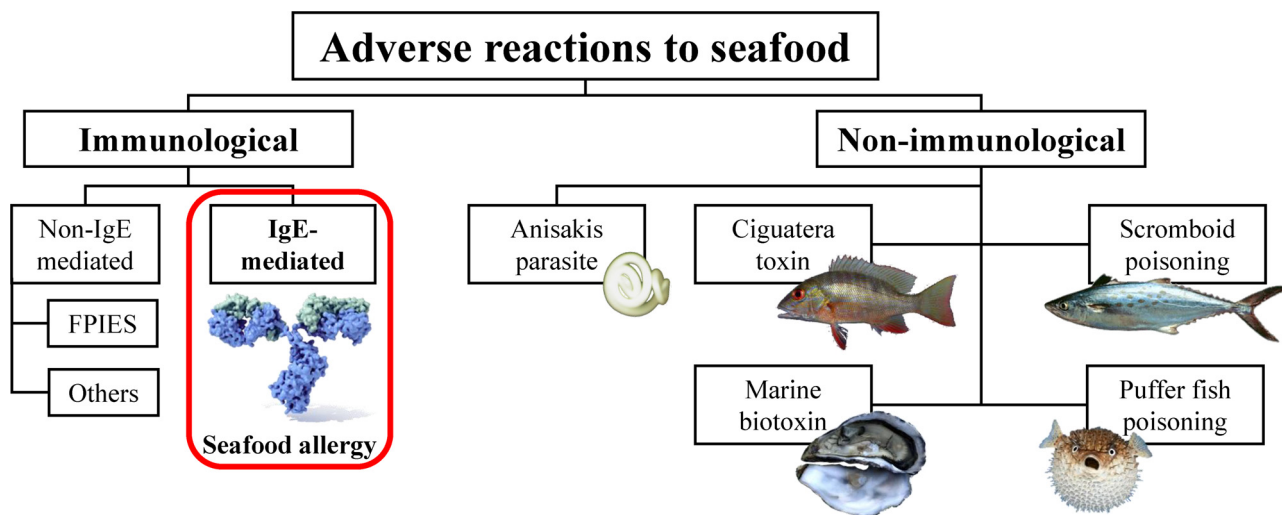


Fig. 2. Overview of adverse reactions to seafood upon ingestion. Some adverse reactions to seafood are non-immunological and/or triggered by substances other than seafood proteins. Immunological reactions are either non-IgE mediated or IgE mediated.

2.1.1. Adverse clinical reactions resembling seafood allergy

2.1.1.1. Scombroid poisoning. Scombroid fish poisoning (also termed histamine poisoning) is an allergy-like reaction that occurs after eating fish that have been improperly refrigerated after capture. Bacteria in the tissue and on the fish skin convert the amino acid histidine into histamine, one of the major mediators of allergic reactions. Enterobacteriaceae, such as *Proteus morganii* and *Klebsiella pneumoniae*, are often implicated. Fish with a high content of red meat, which turns brown upon cooking is commonly involved including mackerel, tuna, herring, sardines, marlin, anchovies and bluefish. Affected fish often have a metallic or peppery taste. Symptoms usually commence within 30 min of eating, and include flushing, hives (urticaria) and nausea and severe episodes may result in anaphylactic reactions.

2.1.1.2. Marine algae toxins. A major cause of adverse seafood reactions is the presence of numerous toxins in fish, crustaceans and molluscs (Visciano et al., 2016; Friedman et al., 2017). The phenomenon of “red tides” is a significant contributor where large blooms of minute toxic dinoflagellates change the colour of the seawater to red, brown, or even green. The toxic algae are taken up by filter feeders such as mussels and oysters, accumulated in the tissue of these invertebrates, and in turn consumed by fish which absorb some of these toxins (ciguatera). Several of these phycotoxins have been identified and characterised; however, the detection in contaminated seafood is often difficult.

2.1.1.3. PSP toxins. PSP (paralytic shellfish poisoning) has been recorded frequently and has accounted for the majority of human fatalities. Minute dinoflagellates (often from the genus *Alexandrium*) produce over 20 related chemical compounds, including the well-characterized neurotoxin saxitoxin, which blocks the neuronal and muscular sodium channels. Symptoms include gastrointestinal signs (nausea, vomiting, diarrhea), but may include a prickly sensation in the fingertips and toes and even muscular paralysis.

2.1.1.4. DSP toxins. Diarrhetic shellfish poisoning (DSP) toxins, which are produced by algae of the Dinophysis or Prorocentrum genera, contain several toxic components including okadaic acid, a potent inhibitor of protein phosphatase enzymes. Chronic exposure to DSP toxins may also promote tumour formation in the digestive tract. Symptoms are mainly gastrointestinal problems such as diarrhea, nausea, vomiting, and abdominal pain.

2.1.1.5. Ciguatera. Ciguatera poisoning is caused by eating fish that has been contaminated by algae-derived toxins. Ciguatoxin, produced by

single-celled dinoflagellates, is a lipid-soluble, heat-stable, and acid-resistant neurotoxin, which concentrates via the food chain in more than 400 different reef fish including Spanish mackerel (family of *Scombridae*) and Red Emperor. Symptoms manifest mainly by gastrointestinal (diarrhea, vomiting), neurologic (temperature reversal, blurred vision) and cardiovascular (drop in blood pressure, heart block) signs. Most affected consumers recover within a few days or weeks with supportive treatment. This fish poisoning causes more human illnesses than any other seafood toxicity and affects up to 50,000 individuals annually.

2.1.1.6. Bacterial and viral contamination. Seafood poisoning outbreaks have frequently been associated with bacterial contamination of shellfish harvested in waters containing inadequately treated sewage. Especially different vibrio strains such as *V. cholerae* or *V. vulnificus* have been implicated as well as *Listeria* and *Salmonella* species. Consumption of raw shellfish, including oysters, often involves small round-structured viruses (SRSVs) and Norwalk virus. Symptoms can occur several hours after consumption and include gastrointestinal symptoms.

2.1.1.7. Anisakis parasite allergy. Allergic reactions after fish consumption can also be triggered by an intestinal fish parasite. The major nematode parasites involved are species of the genus *Anisakis*. This parasite infects many marine fish species as well as seals, whales and dolphins. Cooking of fish at temperatures above 60 °C or storage in industrial freezers for two days is required to kill the parasite. *Anisakis* can cause two major problems in humans: 1) *Anisakis* infection (anisakiasis) can result from eating raw, pickled or undercooked seafood, such as sushi. Infection may cause nausea, vomiting, stomach pain, and sometimes appendicitis, bowel blockage or bleeding. Diagnosis usually requires an endoscopy. However, our immune system destroys the parasite after about three weeks (Baird et al., 2014), 2) *Anisakis* allergy in contrast resembles a typical food allergy. In this case, the allergic reaction is directed towards allergenic proteins of this fish parasite, not to the fish itself. The allergens of *Anisakis simplex* appear to be not destroyed by heat or cooking and so allergic reactions may be triggered by dead parasites, even if the fish has been long frozen or well cooked. The presence of *Anisakis* specific IgE antibody can confirm allergic sensitisation (Nieuwenhuizen and Lopata, 2013, 2014).

2.1.1.8. Seafood intolerance. Adverse reactions to seafood may be caused by pharmacological properties of the ingested seafood,

resulting in intolerance reactions, reflected often with severe headaches. The substances involved include vasoactive amines such as histamine and tyramine, often found in large quantities in canned and pickled fish, and fish autolysates (Biji et al., 2016). However, vasoactive amines can also originate from fresh fish such as herring, tuna, and mackerel, while shellfish contain histamine liberators. In addition, food dyes (e.g. tartrazine) and food additives (e.g. monosodium glutamate, MSG) have also been implicated in adverse reactions.

Other adverse reactions unrelated to seafood are frequently reported including iodine reactivity, glucosamine reactivity and reactions to metabisulfite, but are not discussed in this review.

2.2. Adverse reactions through inhaling or handling seafood

While exposure via ingestion is the major route and trigger for allergic reactions, some sensitive individuals may react to the fumes when seafood is being cooked or processed, and occasionally from touch, such as when cleaning and handling fish (Jeebhay and Lopata, 2012). Symptoms include rhinitis, asthma and skin reaction and can be a problem where exposure is frequent, such as in seafood processing factories, or in some food handlers, such as chefs. These occupational allergies can affect large groups in the workforce, with up to 8% of fish processors (Jeebhay and Lopata, 2012) and even 36% of crustacean processing workers (Thomassen et al., 2016).

The major fish allergen involved seems to be parvalbumin, while tropomyosin seems to be the major crustacean allergen (Lopata and Jeebhay, 2013; Kamath et al., 2014b). Other IgE-binding proteins have also been identified in molluscs and other seafood-associated agents (e.g. Anisakis; proteolytic enzyme), although their exact biological and molecular nature has not been characterised (Nieuwenhuizen et al., 2006; Abdel Rahman et al., 2011). Aerosolised allergens can be identified and quantified using immunological and chemical approaches, detecting levels as low as 10 ng/m³ (Lopata et al., 2005; Kamath et al., 2014b; Thomassen et al., 2016).

2.3. Prevalence and epidemiology of seafood allergy

It is estimated that up to 2.5% of the general population have experienced adverse reactions to seafood. Seafood allergy is more common in adults than in children (Ben-Shoshan et al., 2010; Kamdar et al., 2015) compared to the opposite distribution observed in other food allergies such as egg and milk. The prevalence of seafood allergy varies substantially across regions; however, it appears to dominate in regions where seafood is a staple food.

The highest rate of fish allergy reported for children is 5% of young Finnish (1–4 years old) (Pyrhonen et al., 2009) followed by 2.29% (95% CI, 2.02–2.56) of young Filipino (14–16 years old) populations (Connett et al., 2012) and 2.04% of American adults (95% CI, 1.7–2.38), being the highest reported rate amongst adult populations (McGowan and Keet, 2013). The prevalence of clinically confirmed fish allergy is much lower (Moonesinghe et al., 2016); however, it still affects as high as 0.7% (95% CI, 0.5–1.2) of Swedish (4 years old) (Ostblom et al., 2008) and 0.6% (95% CI, 0.3–1.3) of German (2–6 years old) (Schnabel et al., 2010) children. It is slightly higher for adults with 0.8% (95% CI, 0.2–2.5) of the European population (Burney et al., 2010) confirmed to be sensitised to fish.

The prevalence of shellfish allergy is often higher than that of fish allergy in the general population. Prawn and crab are the major causes of anaphylaxis in both children and adults. Combined crustacean and mollusc allergy reportedly affects as many as 5.5% (95% CI, 4.3–7.1) of French children (5–17 years old) and 9.0% (95% CI, 6.7–11.9) of American adults (Moonesinghe et al., 2016). The prevalence of crustacean allergy alone is considerably higher, with the highest reported prevalence of 10.3% (95% CI, 7.0–14.9) determined among Italian adults who were confirmed positive by Skin Prick Test (SPT) (Burney

et al., 2010). Mollusc allergy was reported in several studies across Europe and Asia (Rance et al., 2005; Osterballe et al., 2009; Lao-araya and Trakultivakorn, 2012; Wu et al., 2012), but its prevalence seems to be less when confirmed using available *in vitro* and *in vivo* tests.

3. Immunoreactivity, biology and biochemistry of fish, crustacean and mollusc allergens

Several allergenic proteins and related isoforms have been identified in commonly consumed seafood species. Seafood allergens are generally stable, water-soluble proteins found in the edible meat. This section provides an overview of the biochemical properties, protein structure and stability and patient serum specific-IgE antibody reactivity of currently identified fish, crustacean and mollusc allergens.

3.1. Fish allergens

Fish allergens have been identified in various parts of the fish, including fish muscle (Arif, 2009), skin, bones (Hamada et al., 2001; Kobayashi et al., 2016b), roe (Perez-Gordo et al., 2008; Shimizu et al., 2009), milt (seminal fluid) (Liu et al., 2016) and blood (Nakamura et al., 2009). The most commonly consumed portion of the fish is muscle tissue, which is frequently heat-treated. Subsequently, heat-stable fish allergens found in muscle are primary focuses of research in the field of fish allergy. However, depending on region and species, other parts of fish are consumed with or without heat treatment and people may be exposed to them during food processing. Therefore, understanding the biochemistry and immunoreactivity of heat-unstable and minor fish allergens is of crucial interest for adequate diagnosis and management of patients with fish allergy.

Members of several protein families have been designated as major or minor allergens in fish, based on their IgE reactivity, and are summarised in Table 1. Parvalbumin, aldolase A, β -enolase, tropomyosin and vitellogenin have been registered as official fish allergens and are recognised by the World Health Organization (WHO)/International Union of Immunological Societies (IUIS) Allergen Nomenclature Sub-Committee (www.allergen.org).

3.1.1. Parvalbumin

The major fish allergen is the small muscle protein parvalbumin (10–13 kDa, pI 4.1–5.2) with a conserved protein structure. The most commonly characterised allergenic fish parvalbumins from cod, salmon and carp were demonstrated to be responsible for 70–100% of allergic reactions to fish and fish products (Sharp et al., 2015; Matricardi et al., 2016). Sensitisation rates to purified and recombinant parvalbumins from different fish species have mainly been elucidated using parvalbumin-specific IgE ELISA or immunoblotting (Bugajska-Schretter et al., 1998; Van Do et al., 2005; Ma et al., 2008; Sharp et al., 2014). Functional reactivity of parvalbumins has been confirmed in some studies by basophil histamine release assays (Bugajska-Schretter et al., 2000). Furthermore, a study by Van Do et al demonstrated positive skin reaction to natural purified parvalbumins from cod, salmon and tuna in 9/10 tested fish-allergic patients (Van Do et al., 2005).

Parvalbumin belongs to one of the largest protein families of food allergens: the calcium-binding proteins (Radauer et al., 2008). It plays a role in the relaxation of muscles after contraction and is a key-protein in fast-contracting vertebrate muscle fibres and other cells in various tissues of all Chordata (Gerday, 1976; Heizmann, 1984; Gillis, 1985; Arif, 2009). Similarly to many other food allergens, parvalbumin is known for extreme heat stability (Lopata and Jeebhay, 2013; Saptarshi et al., 2014), food processing, and enzymatic digestion (Griesmeier et al., 2010a). However, it is suggested that the IgE reactivity of parvalbumin decreases when the tissue is heated to 140 °C (Kubota et al., 2016).

The two distinct phylogenetic lineages of parvalbumin, α and β , differ by isoelectric point; β -parvalbumins contain a greater proportion of acidic amino acid residues and have an isoelectric point (pI) below

Table 1
Seafood proteins with demonstrated IgE reactivity. The presented information has been retrieved from www.allergenonline.org, www.allergome.org as well as the literature. This table lists all currently known seafood proteins, which have been reported as allergens (March 2018). Proteins are listed in alphabetical order. Note: 'X' indicates in which seafood groups the protein was described as an allergen and '-' = no details available.

Protein	Fish	Crustacean	Mollusc	Biological function	Molecular weight	Remark	References
Aldehyde phosphate dehydrogenase	X	-	-	Catalyses oxidation of aldehydes	41 kDa	-	(Das Dorees et al., 2002)
Aldolase A	X	X	-	Enzyme in glycolysis	~40 kDa	-	(Liu et al., 2011b; Kuehn et al., 2013; Kamath et al., 2014a)
Arginine kinase	-	X	X	Energy metabolism	38-45 kDa	Invertebrate equivalent to creatine kinase	(Yu et al., 2003; Garcia-Orozco et al., 2007; Shen et al., 2011, 2012; Chen et al., 2013b)
Collagen	X	-	-	Structural protein	> 100 kDa	-	(Sakaguchi et al., 2000; Hamada et al., 2001; Kobayashi et al., 2016b)
Creatine kinase	X	-	-	Energy metabolism	~40 kDa	Veequivalent to arginine kinase	(Kondo et al., 2006)
β-Enolase	X	X	-	Enzyme in glycolysis	~50 kDa	-	(Liu et al., 2011b; Kuehn et al., 2013; Khanarukombat et al., 2014)
Glyceraldehyde-3-phosphate dehydrogenase	X	-	-	Enzyme in glycolysis	~37 kDa	-	(van der Ventel et al., 2011; Gonzalez-Fernandez et al., 2017)
Hemocyanin	-	X	-	Oxygen transport	77 kDa	Metalloprotein, uses copper	(Giuffrida et al., 2014; Khanarukombat et al., 2014; Zhang et al., 2016)
Myosin light chain 1	-	X	-	Structural protein	17-23 kDa	Small subunit of myosin motor	(Bauermeister et al., 2011)
Myosin light chain 2	-	X	-	Structural protein	17-23 kDa	Small subunit of myosin motor	(Ayuso et al., 2008; Kamath et al., 2014a; Zhang et al., 2015)
Ovary development-related protein	-	X	-	Unknown ovary protein	28 kDa	Found in roe	(Zhu et al., 2016)
Paramyosin	-	-	X	Structural protein	100 kDa	Similar structure as tropomyosin	(Suzuki et al., 2011)
α-parvalbumin	X	-	-	Calcium-binding protein	10-13 kDa	Mainly cartilaginous fish	(Stephen et al., 2017)
β-parvalbumin	X	-	-	Calcium-binding protein	10-13 kDa	Mainly bony fish	(Stephen et al., 2017)
Sarcoplasmic calcium-binding protein	-	X	-	Calcium ion buffering	20-24 kDa	Dimeric; Two main isoforms in crustaceans	(Ayuso et al., 2009; Bauermeister et al., 2011; Chen et al., 2013a)
Triosephosphate isomerase	X	X	X	Glycolytic enzyme	28 kDa	Dimeric enzyme	(Bauermeister et al., 2011; Wang et al., 2011a; Yang et al., 2017)
Tropomyosin	X	X	X	Structural protein	33-39 kDa	-	(Shanti et al., 1993; Daul et al., 1994; Leung et al., 1994; Leung et al., 1998a; Leung et al., 1998b; Reese et al., 2005; Motoyama et al., 2007; Liang et al., 2008; Motoyama et al., 2008; Nakano et al., 2008; Bauermeister et al., 2011; Yadrir et al., 2012; Abramovitch et al., 2013; Liu et al., 2013; Myrset et al., 2013; Koeberl et al., 2014; Gonzalez-Fernandez et al., 2016)
Troponin C	-	X	-	Structural protein	~20 kDa	Subunit of troponin trimer	(Bauermeister et al., 2011; Kalyanasundaram and Santiago, 2015)
Troponin I	-	X	-	Structural protein	~30 kDa	Subunit of troponin trimer	WHO/IUIS
Vitellogenin	X	X	-	Yolk protein	18 kDa	Found in roe	(Mäkinen-Kiljunen et al., 2003; Perez-Gordo et al., 2008; Khanarukombat et al., 2014; Zhu et al., 2016)

4.8 (Pechere et al., 1971; Arif, 2009). Furthermore, α and β lineages of parvalbumins have characteristic amino acids at specific positions (A-14, L-16, C-19, F-67, Q-69, F-79 for β -parvalbumins) (Beale et al., 2009). β -parvalbumins are predominantly expressed in bony fish and are known to cause IgE-mediated allergic reactions in humans (Jenkins et al., 2007). In cartilaginous fish, on the other hand, only α -parvalbumins have been found, for which a lower IgE-reactivity is proposed. Koyama et al demonstrated reduced IgE binding to extracts from several shark species compared to IgE binding to bony fish extracts in 38 Japanese fish-allergic subjects. However, the role of α -parvalbumin in lower allergenicity of cartilaginous fish has not been demonstrated (Koyama et al., 2006). To our knowledge, there has only been one report showing IgE binding to purified α -parvalbumin, with 15 fish-allergic patients sera reacting to red stingray parvalbumin (Cai et al., 2010).

3.1.2. Aldolase A and β -enolase

Aldolase A (40 kDa) and β -enolase (47–50 kDa), are essential glycolytic enzymes and have been identified as important allergens in muscle tissue of bream (Liu et al., 2011b), cod, salmon and tuna (Kuehn et al., 2013). Aldolase was first identified as a fish allergen in Pacific salmon in 2009 (Nakamura et al., 2009). Enolase was detected as a fish allergen in bream in 2011 by Liu et al., where IgE binding to this protein was demonstrated in 10 individuals with confirmed bream allergy by immunoblotting (Liu et al., 2011b). In a study by Kuehn et al. from 2013, aldolase A and β -enolase were further characterised as fish allergens using specific IgE ELISA and, in selected cases, basophil degranulation assays. This study included a total of 62 individuals with fish allergy from Luxembourg and France. Demonstrated sensitisation rates were 13–37% to aldolase A and 14–56% to β -enolase from different fish species (Kuehn et al., 2013).

Aldolase and enolase were found to be sensitive to heat treatment (Kuehn et al., 2013) and seem to be less resistant to food processing than parvalbumin. However, this has not been investigated comprehensively.

3.1.3. Tropomyosin

The structural protein tropomyosin (33–39 kDa), a pan-allergen for shellfish, has been registered as an allergen for only one fish species by WHO/IUIS, Mozambique tilapia (*Oreochromis mossambicus*, WHO/IUIS Ore m 4). Tropomyosin from fish has been reported as an allergen in only one study (Liu et al., 2013) since 2012. It was identified by immunoblotting and specific IgE ELISA using sera from 10 individuals with confirmed allergy to tilapia. However, a more recent study from 2018 has further highlighted the importance of recognising fish tropomyosin as an allergen by demonstrating IgE-reactivity to cod and albacore tropomyosin in 10/19 patients (Gonzalez-Fernandez et al., 2018).

3.1.4. Other fish allergens

Other proteins that are potential allergens have been identified in various fish species. Collagen was first described as a fish allergen in a study by Hamada et al in 2001, where it was purified from muscle tissue of tuna and demonstrated IgE binding in 5/8 patients' sera. In another study by Kobayashi et al, IgE reactivity to collagen from Pacific mackerel skin was shown in 18/36 patients (50%) with confirmed allergy to fish by specific IgE ELISA (Hamada et al., 2001; Kobayashi et al., 2016b). Allergens identified in parts of fish other than muscle and skin include vitellogenin as the major allergen in fish roe (caviar) (Perez-Gordo et al., 2008; Shimizu et al., 2009), protamine as the major allergen in milt (seminal fluid) (Liu et al., 2016), and serum albumin as a potential allergen in blood (Nakamura et al., 2009). Some of these proteins are also expressed in muscles or present in muscle tissue due to contamination.

Other potential fish allergens include aldehyde phosphate dehydrogenase (Das Soares et al., 2002), triosephosphate isomerase (Wang

et al., 2011a), glyceraldehyde-3-phosphate dehydrogenase (van der Ventel et al., 2011; Gonzalez-Fernandez et al., 2017), and creatine kinase (Liu et al., 2011b; Larco-Rojas et al., 2017) as well as several unidentified proteins of different molecular weight (4, 7, 18, 25, 40, 42, 45, 46, and 51 kDa) (Kelso et al., 1996; Asero et al., 1999; Rosmilah et al., 2005; Ebo et al., 2010). However, the allergenic relevance of these molecules is still to be determined, and they will not be discussed in this review.

3.2. Crustacean and mollusc allergens

Allergens in various crustacean and mollusc species are mainly found in the edible portions of meat. Several crustacean allergens have been identified over the past two decades, due to the advances in protein identification and allergen characterisation techniques. Crustacean allergens are proteins found mostly in the muscle tissue, cephalothorax or in roe. Their physiological functions are involved in muscle movement and energy metabolism. Prawns are a commonly consumed crustacean species and more than six prawn allergens have been identified (Table 1). Several allergens have also been identified from edible parts of mollusc species. Three allergenic proteins: tropomyosin, arginine kinase and triosephosphate isomerase, have also been identified in other shellfish groups.

3.2.1. Tropomyosin

Tropomyosin is considered the major invertebrate pan-allergen found in all edible crustacean species. Tropomyosin-specific IgE quantification in shrimp-allergic patients has demonstrated that 72–98% of patients showed positive IgE binding to purified tropomyosin (Ayuso et al., 2010; Bauermeister et al., 2011; Gamez et al., 2011). Epitope mapping studies have shown the presence of eight IgE binding epitopes (Reese et al., 2005) that are capable of binding and cross-linking IgE on basophils derived from shrimp-allergic patients (Abramovitch et al., 2013, 2017).

Tropomyosin belongs to a family of highly conserved actin-binding proteins involved in muscle contraction in vertebrates and invertebrates (Reese et al., 1999). Tropomyosin exists as a complex with troponin and is involved in muscle contractile function by interacting with actin and myosin (Nevzorov and Levitsky, 2011; Oguchi et al., 2011; Behrmann et al., 2012). It is present in muscle (skeletal, cardiac and smooth), brain, platelets, fibroblasts, and many other non-muscle cells. The physiological state of tropomyosin is a highly stable, α -helical coiled-coil homo-dimeric protein. The stability of the coiled-coil structure of tropomyosin is a function of the interaction of repeated sequence patterns of seven amino acids over the entire length of the protein, with a hydrophobic amino acid in every first and fourth position (Lees-Miller and Helfman, 1991). This form of secondary and tertiary structure imparts structural stability to tropomyosin and it is therefore able to withstand heat and high-pressure processing and retain its allergenicity (Kamath et al., 2013; Kamath et al., 2014a). Several studies have investigated the effects of thermal and enzymatic digestion on tropomyosin. Although tropomyosin is highly heat-stable, studies have demonstrated an increase in antibody binding to tropomyosin after heat treatment (Kamath et al., 2013; Kamath et al., 2014a). Simulated digestion studies have shown that tropomyosins are susceptible to gastric pepsin and trypsin degradation (Liu et al., 2011a; Gamez et al., 2015).

Tropomyosin is the major crustacean allergen, identified and registered by WHO/IUIS as an allergen in 13 different species. In crustaceans, the fast twitch and the slow twitch isoforms were identified in the tail muscles and the pincer muscles, respectively (Motoyama et al., 2007). Tod p 1 was the first major mollusc allergen submitted to WHO/IUIS and was isolated from squid (*Todarodes pacificus*). The allergen is a 38 kDa, heat-stable protein and was later identified as tropomyosin after amino acid sequencing and alignment with tropomyosin from other organisms (Miyazawa et al., 1996). Shortly after, other tropomyosins were identified in other groups of mollusc species such as oyster (Ishikawa et al., 1997), and snail (Ishikawa et al., 1998a).

3.2.2. Arginine kinase

Arginine kinase (AK) was characterised in shrimps as a clinically relevant allergen after tropomyosin. Subsequently, AK has been identified and characterised in crabs, lobster, prawns, and octopus. Similar to tropomyosin, the diagnostic utility of AK has been investigated for shrimp allergy, with 10–51% of shrimp-allergic patients eliciting positive IgE binding (Ayuso et al., 2010; Bauermeister et al., 2011; Giuffrida et al., 2014; Pascal et al., 2015).

Arginine kinase is involved in the regulation of cellular ATP levels and is an essential enzyme in the energy metabolism of invertebrates. It catalyses the transfer of a phosphate group from ATP to L-arginine. Phosphoarginine is the most important phosphagen in invertebrates, synonymous in function to phosphocreatine in vertebrates. Sequence analysis of AK and creatine kinase concluded that they are derived from a common origin (Uda et al., 2006), with the homologs displaying a sequence identity of < 39%. AK is widely distributed throughout invertebrates and generally found as a monomer, although some dimeric AK have been observed (Uda et al., 2006). It is highly concentrated in muscle tissue (Abdel Rahman et al., 2011) and binding to the muscle protein actin has previously been demonstrated (Reddy et al., 1992).

Epitope mapping studies have shown the presence of seven IgE binding epitopes and confirmed the role of structural stability for IgE binding (Ayuso et al., 2010; Yang et al., 2015). Accordingly, loss of IgE reactivity upon heat-treatment was demonstrated (Chen et al., 2013b). AK is a heat labile protein, with previous studies showing reduced allergenicity after thermal and pH treatment (Kamath et al., 2014a). It has a molecular weight of 38–45 kDa and a pI of approximately 6 (Jarilla et al., 2014). AK the second crustacean allergen discovered and has been described in black tiger prawn (*Penaeus monodon*), white-leg shrimp (*Litopenaeus vannamei*), red swamp crayfish (*Procambarus clarkii*) (Chen et al., 2013b), and North sea shrimp (*Crangon crangon*) (Bauermeister et al., 2011). AK has also been described as a mollusc allergen, first discovered in octopus (*Octopus fangsiao*) (Shen et al., 2012). The amino acid sequence shares 54% identity between crustaceans and molluscs. It is also well-known for its role in house dust mite allergy (Abdel Rahman et al., 2011), as well as insect allergy.

3.2.3. Myosin light chain

Myosin light chain (MLC) was identified as an allergen in white-leg shrimp (Lit v 3) (Ayuso et al., 2008). It is considered a minor allergen, with the frequency of allergic sensitisation ranging from 19 to 55% (Bauermeister et al., 2011; Pascal et al., 2015). IgE binding studies indicate that recognition of MLC is higher in adults compared to children, using heated shrimp extract.

Myosin light chains form part of the large myosin macromolecular complex that make up the functional muscle protein structure (actin-binding molecular motor). The multimetric protein consists of two heavy chains (~200 kDa) and four light chains (~18–20 kDa). The light chains belong to the calmodulin-like protein family (along with parvalbumin) that bind Ca^{2+} through their EF-hand domain. There are two classes of myosin light chain, which are referred to as the essential light chain (MLC1) and the regulatory light chain (MLC2) (also referred to as α and β chains). Like many food allergens, crustacean MLCs are heat and pH stable (Zhang et al., 2015). Studies have shown that IgE recognises primarily conformational rather than linear epitopes of MLC1 (Zhang et al., 2015).

MLC1 (allergen number 5, WHO/IUIS) has been characterised as an allergen in North Sea shrimp (Bauermeister et al., 2011) and red swamp crayfish (Zhang et al., 2015). MLC2 (allergen number 3, WHO/IUIS) has been characterised as an allergen in American lobster (*Homarus americanus*), black tiger prawn (Kamath et al., 2014a), and white-leg shrimp (Ayuso et al., 2008).

3.2.4. Sarcoplasmic calcium-binding protein

Sarcoplasmic calcium-binding protein (SCBP), was first identified as a shellfish allergen in Black tiger prawn (Pen m 4) and Pacific white-leg shrimp (Lit v 4) (Shiomi et al., 2008; Ayuso et al., 2009). Although

considered a minor allergen, SCBP elicits 29–50% positive IgE binding among shrimp-allergic patients. Compared to other shellfish allergens, SCBP contains only three IgE binding epitopes (Ayuso et al., 2010). However, it was demonstrated that SCBP contains conformational type IgE epitopes that may partially be dependent on calcium binding (Mori et al., 2013). Interestingly, IgE recognition was found to be higher in children than in adults, and with a greater relevance in predicting clinical reactivity to shrimps in combination with tropomyosin-specific IgE (Ayuso et al., 2009; Pascal et al., 2015).

Sarcoplasmic calcium-binding proteins belong to the EF-hand calcium-binding protein family that contain helix-loop-helix motifs to bind calcium ions (Ca^{2+}). They are found exclusively in invertebrates and are thought to act as a functional analogue to vertebrate parvalbumin. Like parvalbumin they have a role in the buffering of cytosolic calcium in muscles; however, SCBPs are larger proteins (20–24 kDa compared to 12 kDa) that form dimers, so may have other functions which are not yet known (White et al., 2011). The sequence identity varies greatly between species and multiple isoforms have been characterised. Crustacean SCBPs have two peptides (Wnuk and Jauregui-Adell, 1983; Takagi et al., 1984), subunits α and β , which can combine as either a homodimer or heterodimer isotype (α_2 , $\alpha\beta$, β_2 ; SCBP-I, -II, and -III, respectively). Furthermore, multiple variants have also been reported (White et al., 2011). SCBPs contain four EF-hand domains, of which two or three retain the ability to bind either Ca^{2+} only, or mixed $\text{Ca}^{2+}/\text{Mg}^{2+}$, depending on the domain. Binding of Ca^{2+} occurs cooperatively (Wnuk and Jauregui-Adell, 1983), with marked structural changes occurring after binding (Wnuk et al., 1981; Christova et al., 2000). SCBPs are heat resistant and stable under acid/alkali treatment (Chen et al., 2013a).

SCBP has been described as an allergen in a number of crustacean species including black tiger prawn (Shiomi et al., 2008), white-leg shrimp (Ayuso et al., 2009), North Sea shrimp (Bauermeister et al., 2011), red swamp crayfish (*P. clarkii*), and narrow-clawed crayfish (*Pontastacus leptodactylus*) (Chen et al., 2013a).

3.2.5. Troponin

Troponin has been identified as a minor crustacean allergen with IgE sensitisation rates ranging from 12 to 29% (Bauermeister et al., 2011; Pascal et al., 2015). Troponins bind with tropomyosin to the thin filament to regulate the contraction of striated muscle. The troponin complex is comprised of three subunits, Troponin C, I and T, of which Troponin C and I have been shown to be IgE-reactive. Troponin C binds Ca^{2+} to regulate activation in response to Ca^{2+} efflux. It belongs to the large family of calcium-binding proteins along with myosin light chain, parvalbumin and calmodulin. It contains four EF-hand motifs, of which two contain functional Ca^{2+} -binding loops in crustaceans (Tanaka et al., 2013). There are three main Troponin C isoforms in crustaceans (Garone et al., 1991), with an additional eight isoforms detected using genetic techniques (Chao et al., 2010). Troponin C has been described as an allergen in North sea shrimp (Bauermeister et al., 2011), black tiger prawn (Kalyanasundaram and Santiago, 2015), and American lobster (Chao et al., 2010). The Troponin I subunit inhibits muscle contraction by binding to actin and preventing the actin-myosin interaction. Troponin I has been identified as a crustacean allergen in narrow-clawed crayfish (WHO/IUIS: Pon 1 7).

3.2.6. Triose phosphate isomerase

Triose phosphate isomerase (TIM) has been characterised as a shrimp and octopus allergen (Bauermeister et al., 2011; Kamath et al., 2014a; Yang et al., 2017). It is considered a minor allergen with the frequency of IgE sensitisation ranging from 15 to 23%. It is a 26–29 kDa enzymatic protein that exists as a dimer, and its primary function is in glucose energy metabolism. Apart from crustacean and mollusc species, TIM has been identified as an allergen in cockroach and plants (Hoppe et al., 2006; Chuang et al., 2010). The cross-reactivity among these species due to TIM has not been investigated in detail.

3.2.7. Paramyosin

Paramyosin, a major structural component of thick filaments in invertebrate muscle, was identified as another allergen in abalone (*Haliotis discus discus*) (Suzuki et al., 2011, 2014) and Pacific oyster (*Crassostrea gigas*) (Nugraha et al., 2018). The discovery of allergenic paramyosin in mollusc species was not surprising since this protein has been confirmed as a major allergen in other invertebrates such as house dust mite (Lee et al., 2004) and Anisakis (Perez-Perez et al., 2000). Paramyosin is a significant component of the bivalve myofibril, making up 38–48% of the white adductor muscle and 15–30% of the red adductor muscle and 9–14% on the myofibril of the mantle muscle of squid and the foot muscle of turban shell (Kantha et al., 1990). The structure of paramyosin is similar to that of tropomyosin, and it is sometimes referred to as “water-insoluble tropomyosin” or “tropomyosin-A”. The molecule is comprised of two α -helical coiled-coil proteins with short non-helical extensions at both the N- and C-termini (Kantha et al., 1990). Although the cross-reactivity of paramyosin and tropomyosin has been demonstrated, the IgE of allergic patients did not react with paramyosin from heated abalone extract, suggesting that the observed cross-reactivity may not be clinically significant, as abalone is generally cooked before consumption (Suzuki et al., 2011). Paramyosin is a heat and pH labile protein and aggregates during extraction in a buffer containing a low salt concentration.

3.2.8. Other crustacean and mollusc allergens

Several other proteins found in various crustacean species have been identified as potential allergens, based on IgE recognition from shellfish-allergic patient sera. Hemocyanin has been identified as an allergen in shrimps (Giuffrida et al., 2014; Khanaruksombat et al., 2014) and crabs (Zhang et al., 2016). It is a high molecular weight multi-subunit protein that is mostly found in the hemolymph or hepatopancreatic regions. It consists of an oxygen transport protein complex that interacts with copper ions, giving it a characteristic blue-green colour. A 2014 case study reported anaphylaxis to shrimp caused by hemocyanin and the utility of hemocyanin for shrimp allergy diagnostics was investigated along with other major shrimp allergens. Notably, hemocyanin has been implicated in shellfish-mite or shellfish-insect cross-reactivity (Giuffrida et al., 2014; Srinroch et al., 2015; Kamath et al., 2017). The clinical implications of cross-reactivity due to hemocyanin has yet to be investigated in detail. Other potential crustacean allergens are fructose bis-phosphate aldolase and titin, which require further investigation to confirm IgE reactivity (Kamath et al., 2014a).

While only tropomyosin, paramyosin and arginine kinase have been confirmed as allergens in molluscs, additional proteins may cause an allergic reaction. Indeed, many unknown proteins have been reported to elicit hypersensitivity. The common whelk (*Buccinum datum*) was found to have several IgE binding proteins with molecular weights of 40, 71, and 82 kDa. These proteins were heat-stable but demonstrated rapid degradation in simulated intestinal fluid and moderate degradation in simulated gastric fluid. Similarly, various proteins from the brown garden snail (*Helix aspersa*) were found to bind IgE from allergic patient sera (Asturias et al., 2002). Recently 23 unreported allergens were identified in the Pacific oyster using biochemical and computational tools in addition to IgE antibody reactivity (Nugraha et al., 2018).

4. Allergen sequence similarity - molecular and clinical cross-reactivity

4.1. Fish allergen sequences and cross-reactivity

Most fish express several different isoforms of the major fish allergen parvalbumin (up to 5), primarily of the β -lineage, with a large molecular diversity most likely being responsible for differential clinical reactivity resulting in mono- or multiple-sensitivity (Kuehn et al.,

2014). It is generally accepted that fish allergy sufferers have an approximately 50% chance of being cross-reactive to another fish species (Sicherer and Sampson, 2010; Kobayashi et al., 2016c) and mono-sensitivity to only one type of species such as tilapia and salmon has been reported (Ebo et al., 2010; Kuehn et al., 2011). Different linear IgE-binding epitopes of parvalbumin from five fish species (Atlantic cod, Atlantic salmon, Baltic cod, common carp, and chub mackerel) have been characterised and probably contribute to species-specific reactivity (Elsayed and Apold, 1983; Untersmayr et al., 2006; Yoshida et al., 2008; Perez-Gordo et al., 2012, 2013; Sharp and Lopata, 2014). However, the variation of linear IgE-binding epitopes is not sufficient to explain all the different IgE-binding profiles demonstrated for species of mackerel and tuna (Yoshida et al., 2008). Multiple parvalbumin proteins and aggregates have been identified in fish as monomers, dimers and oligomers (Saptarshi et al., 2014). Most fish parvalbumins have been identified by immunoblotting, utilising the commercial anti-frog parvalbumin monoclonal antibody PARV-19 (Chen et al., 2006; Lim et al., 2008; Beale et al., 2009; Gajewski and Hsieh, 2009; Weber et al., 2009). Polyclonal antibodies against parvalbumin from a range of different fish species, such as barramundi, basa/catfish, carp, cod, pilchard, and salmon, have recently been used successfully in the identification of parvalbumin, including in some Asian fish species (Swoboda et al., 2002b; Lee et al., 2011; Sharp et al., 2015; Ruethers et al., 2018).

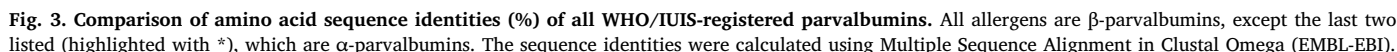
Parvalbumin was first described as an allergen in the early 1970s in Baltic cod. This allergen was subsequently named Gad c 1 and as of March 2018, β -parvalbumins from 13 fish species were officially registered by WHO/IUIS and named accordingly (Table 2). The database, maintained by the United States National Library of Medicine (www.ncbi.nlm.nih.gov/pmc) listed 156 publications about the ‘fish allergen parvalbumin’ as of March 2018. Non-fish parvalbumins are WHO/IUIS-recognised allergens from frog (α - and β -isoform) and chicken (α -isoform) with reported cross-reactivity between chicken and fish parvalbumins (Kuehn et al., 2016).

Parvalbumins and their allergenicity have been well investigated for many bony fish species consumed in West Europe and North America. Those studies show large amino acid sequence diversity between species such as cod, carp, and salmon (Kuehn et al., 2014; Sharp and Lopata, 2014). The diversity of WHO/IUIS-recognised parvalbumin isoforms is demonstrated in Fig. 3. Notably, parvalbumin isoforms from the same species often share less than 68% amino acid sequence identity as shown for barramundi and rainbow trout, adding complexity to the detection of allergenic parvalbumins. Among all β -parvalbumins, the sequence identities range from 46 to 99%, with the lowest identity between the respective isoforms from Baltic cod and rainbow trout, suggesting the possibility of limited cross-reactivity. Parvalbumins of the α -lineage share only 43–60% amino acid sequence identity with β -parvalbumins. Human α -parvalbumin (UniProt: P20472) has a sequence identity with all WHO/IUIS-registered α - and β -parvalbumins of 44–58% and 68–79%, respectively. α -parvalbumins have been reported as allergens in cartilaginous fish (Stephen et al., 2017); however, clinical relevance is yet to be determined. In addition, the vast majority of the more than 32,300 fish species have never been investigated for their allergens (Sharp and Lopata, 2014).

The allergenicity of any given fish species is dependent on two factors; the allergenicity of the proteins (in this case parvalbumin), and on the amount of the allergens present in the tissue (Griesmeier et al., 2010b; Kuehn et al., 2010; Kobayashi et al., 2016a). A study by Kuehn et al. (Kuehn et al., 2014) demonstrated that the parvalbumin content may differ up to 48 fold between fish species (0.2–11.2 mg of parvalbumin per gram of tissue), dependant on the size of the specimen, the species and also the type of fish (sedentary or migratory type). Furthermore, the parvalbumin content within one species can differ up to 7.4-fold between different muscle parts, as demonstrated for the Mediterranean flying fish (Kobayashi et al., 2016d). Generally, higher parvalbumin content is observed in dorsal and rostral compared to ventral and caudal parts of fish muscle, respectively. Furthermore, parvalbumin

Table 2
WHO/IUIS registered fish allergens. Fish allergens registered with the WHO/IUIS (www.allergen.org) are listed in alphabetical order of given allergen names. Note: '*' reference is not directly linked to the WHO/IUIS allergen entry. MW = molecular weight in kDa and 's' = no details available.

Fish species	Allergen	Protein type	MW	Genbank protein accession number of isoforms		References
				.0101 .0102	.0201 .0202	
Atlantic herring	<i>Clupea harengus</i>	β -parvalbumin	12	CAQ72970	CAQ72971	CAQ72972 (Swoboda et al., 2013)
Common carp	<i>Cyprinus carpio</i>	β -parvalbumin	12	CAC83658	CAC83659	– (Bugajska-Schretter et al., 2000)
Baltic cod	<i>Gadus callarias</i>	β -parvalbumin	12	P02622	–	– (Bugajska-Schretter et al., 1998)
Atlantic cod	<i>Gadus morhua</i>	β -parvalbumin	12	AAK63086 CAM56785	AAK63087 CAM56786	– (Van Do et al., 2003)
	Gad m 2	β -enolase	47	B3A0L6	–	– (Kuehn et al., 2013)*
	Gad m 3	Aldolase A	40	P86980	–	– (Kuehn et al., 2013)*
Barramundi	<i>Lates calcarifer</i>	β -parvalbumin	12	AAV97933/AHW83198	AAT45383	– (Sharp et al., 2014)
Whiff	<i>Lepidorhombus whiffiagonis</i>	β -parvalbumin	12	CAP17694	–	– (Griesmeier et al., 2010a)*
Chum salmon (roe)	<i>Oncorhynchus keta</i>	β -prime-component of vitellogenin	18	BAJ07603	–	– (Shimizu et al., 2014)*
Rainbow trout	<i>Oncorhynchus mykiss</i>	β -parvalbumin	12	P86431	P86432	– (Kuehn et al., 2010)*
Mozambique tilapia	<i>Oreochromis mossambicus</i>	Tropomyosin	33	AFV53352	–	– (Liu et al., 2013)*
Indian Mackerel	<i>Rastrelliger kanagurta</i>	β -parvalbumin	11	ANW10058.1	–	– (Ruethers et al., 2018)*
Atlantic salmon	<i>Salmo salar</i>	β -parvalbumin	11	CAA66403	–	– (Lindstrom et al., 1996)*
	Sal s 2	β -enolase	50	ACH70931 ACH70932	–	– (Kuehn et al., 2013)*
	Sal s 3	Aldolase A	40	ACH70901	–	– (Kuehn et al., 2013)*
Pacific pilchard	<i>Sardinops sagax</i>	Parvalbumin	12	CAQ68366	–	– (Beale et al., 2009)*
Ocean perch	<i>Sebastes marinus</i>	Parvalbumin	11	CAQ72968	CAQ72969	– (Kuehn et al., 2010)*
Yellowfin tuna	<i>Thunnus albacares</i>	β -parvalbumin	11	CAQ72967	–	– (Kuehn et al., 2010)*
	Thu a 2	β -enolase	50	P86978	–	– (Kuehn et al., 2013)*
	Thu a 3	Aldolase A	40	P86979	–	– (Kuehn et al., 2013)*
Swordfish	<i>Xiphias gladius</i>	β -parvalbumin	12	CAR48256	–	– (Griesmeier et al., 2010b)*



Collagen has been comprehensively investigated as an allergen in the Japanese population and was identified as a highly cross-reactive pan-allergen along with parvalbumin (Kobayashi et al., 2016b). All other fish allergens have only been investigated in a limited number of species and their cross-reactivity is poorly understood. Vitellogenin and tropomyosin are the only WHO/IUIS registered allergens in chum salmon and Mozambique tilapia, respectively. For vitellogenin, an IgE cross-reactivity was demonstrated, but the clinical relevance remains unknown (Shimizu et al., 2009). Aldolase A and β -enolase from Atlantic cod and Atlantic salmon have been well characterised, and may be useful for the identification of patients with limited cross-reactivity (Kuehn et al., 2013; Sorensen et al., 2017).

Crustacean allergens exhibit a high degree of conservation and amino acid sequence identity among their member species as well as between molluscs, insects, mites and other invertebrates. This is the primary reason for clinical and immunological cross-reactivity among these species due to the presence of shared IgE epitopes. Moreover, the high frequency of cross-reactivity presents a challenge in identifying the primary sensitising source during routine allergy diagnosis and differentiation from other cross-reacting allergens. Tropomyosin is the major allergen implicated in clinical and IgE antibody cross-reactivity, and several studies have investigated the role of crustacean tropomyosin in cross-reactivity to other invertebrate sources (Table 3). Cross-reactivity among crustaceans and molluscs is frequently observed due to tropomyosin's potential to sensitise via occupational exposure in addition to exposure by ingestion (Goetz and Whisman, 2000; Zhang et al., 2006). Cross-reactivities between crustaceans and house dust mites, as well as insects, have been extensively investigated due to their high clinical impact on sensitised individuals upon accidental exposure and subsequent allergic reactions to the seafood allergens (Ayuso et al., 2002; Arlian et al., 2009; Boquete et al., 2011; Wang et al., 2011b, Lopez-Matas et al., 2016; Kamath et al., 2017). Tropomyosin has a highly

Besides tropomyosin, arginine kinase has been implicated in cross-reactivity to moths, spiders, and silkworms (Binder et al., 2001; Liu et al., 2009; Bobolea et al., 2011). In addition, hemocyanin may be a potential cross-reactive allergen identified so far as an allergen in crustaceans, dust mites, cockroaches and molluscs (Giufrida et al., 2014; Khanaruksombat et al., 2014; Khurana et al., 2014; Kamath et al., 2017).

The molecular heterogeneity of allergenic mollusc tropomyosins (70–97% sequence identity, see Fig. 4) poses a challenge for clinicians to identify the species responsible for allergic reactions. There are many cases of patients with IgE reactivity to only a very narrow range of mollusc species (Carrillo et al., 1992; Jimenez et al., 2005; Martin-Garcia et al., 2007; Rodriguez-Del Rio et al., 2009). Another study demonstrated the utility of this diversity for phylogenetic analysis of molluscs (Wang et al., 2011c). There are 56 tropomyosins from 41 different mollusc species listed by UniProt (www.uniprot.org), while only five are registered as allergens with the WHO/IUIS (Table 4). Few studies have comprehensively investigated cross-reactivities among mollusc species. The largest studies demonstrated serum IgE reactivity to ten cephalopods, seven bivalves, and four gastropods for ten shellfish-allergic patients (Motoyama et al., 2006; Emoto et al., 2009). In a clinical study with 17 patients, only ten were co-sensitised to abalone

Table 3
WHO/IUIS registered crustacean allergens. Crustacean allergens registered with the WHO/IUIS (www.allergen.org) are listed in alphabetical order of given allergen names. Note: '*' reference is not directly linked to the WHO/IUIS allergen entry. MW = molecular weight in kDa and '-' = no details available.

Crustacean species	Allergen	Protein type	MW	Genbank protein accession number of isoform		References
				.0101	.0102	
Common name unavailable	<i>Archaeopotamobius sibiricus</i>	Arc s 8	28	CAD29196	-	-
Flowery crab	<i>Charybdis feriatus</i>	Cha f 1	34	AAF35431	-	(Leung et al., 1998b)*
North Sea shrimp	<i>Crangon crangon</i>	Gra c 1	38	ACR43473	-	(Bauermeister et al., 2011)*
		Gra c 2	45	ACR43474	-	(Bauermeister et al., 2011)*
		Gra c 4	25	ACR43475	-	(Bauermeister et al., 2011)*
		Gra c 5	18	ACR43477	-	(Bauermeister et al., 2011)*
Chinese mitten crab	<i>Eriocheir sinensis</i>	Gra c 6	21	ACR43478	-	(Bauermeister et al., 2011)*
		Gra c 8	28	ACR43476	-	(Bauermeister et al., 2011)*
		Eri s 2	28	AA073305	-	(Zhu et al., 2016)
		Hom a 1	-	AAC48287	AAC48288	(Leung et al., 1998b)
American lobster	<i>Homarus americanus</i>	Hom a 3	~23	EH115965	-	-
		Hom a 6	~20	P29291	-	-
		Lit v 1	36	ACB38288	-	-
White-leg shrimp	<i>Litopenaeus vannamei</i>	Lit v 2	-	ABI98020	-	(Garcia-Orozco et al., 2007)*
		Lit v 3	20	ACC76803	-	(Ayuso et al., 2008)*
		Lit v 4	20	ACM89179	-	(Ayuso et al., 2009)*
		Mac r 1	37	ADC55380	-	(Yadzir et al., 2012)*
Giant freshwater prawn	<i>Macrobrachium rosenbergii</i>	Mel l 1	38	AGF86397	-	(Koeberl et al., 2014)*
King prawn	<i>Melicerius latissulcatus</i>	Met e 1	34	AAA60330	-	(Leung et al., 1994)
Greasyback shrimp	<i>Metapenaeus ensis</i>	Pan b 1	37	CBY17558	-	(Myrset et al., 2013)*
Northern shrimp	<i>Pandalus borealis</i>	Pan s 1	34	AAC38996	-	(Leung et al., 1998b)
Spiny lobster	<i>Panulirus stimpsoni</i>	Pen a 1	36	AAZ76743	-	(Daul et al., 1994)
Brown shrimp	<i>Penaeus aztecus</i>	Pen i 1	34	-	-	(Shanti et al., 1993)
Indian prawn	<i>Penaeus indicus</i>					

(continued on next page)

Table 3 (continued)

Crustacean species	Allergen	Protein type	MW	Genbank protein accession number of isoform		References
				.0101	.0102	
Black tiger prawn	Pen m 1	Tropomyosin	38	AAX37288,BAF47262,ADM34184	-	(Motoyama et al., 2007; Kamath et al., 2014a)*
	Pen m 2	Arginine kinase	40	AA015713	-	(Yu et al., 2003)
	Pen m 3	Myosin light chain 2	-	ADVI7342 ADM34185	-	(Kamath et al., 2014a)*
	Pen m 4	Sarcoplasmic calcium binding protein	-	ADVI7343	-	-
	Pen m 6	Troponin C	-	ADVI7344	-	(Kalyanasundaram and Santiago, 2015)*
	Pon 1 4	Sarcoplasmic calcium-binding protein	~24	P05946	-	-
Narrowclawed crayfish	Pon 1 7	Troponin I	~30	P05547	-	-
Blue swimmer crab	Por p 1	Tropomyosin	39	AGE44125	-	(Abramovitch et al., 2013)*

and snail as shown by RAST (Lopata et al., 1997).

Tropomyosins from molluscs share only 55–65% amino acid sequence identity with allergenic tropomyosins from crustacean, insects, mites, and fish (Fig. 4). However, it was demonstrated that tropomyosin is responsible for clinical cross-reactivity between molluscs, crustaceans, cockroach, and house dust mite (Leung et al., 1996; Bessot et al., 2010).

The other reported mollusc allergens have only been identified and characterised in abalone (paramyosin) (Carrillo et al., 1992; Jimenez et al., 2005; Martin-Garcia et al., 2007; Rodriguez-Del Rio et al., 2009) and octopus (arginine kinase and triosephosphate isomerase) (Shen et al., 2012; Yang et al., 2017) and the cross-reactivity of these allergens has only been investigated in crustaceans. However, a recent study demonstrated the presence of multiple allergens in Pacific oyster that are common in various allergen sources including fish, mites, pollen, plant, and fungi (Nugraha et al., 2018). This suggests the possibility of cross-reactivity between molluscs and other allergen sources.

4.4. Cross-reactivity between fish, crustacean, and mollusc

Recent studies on the identification and characterisation of novel allergens have identified a wide range of cross-reactive allergens. Interestingly, several allergens found independently in fish, crustacean or mollusc species originate from the same protein family or share a similar structure and function. Although there is lack of evidence proving their role in clinical cross-reactivity among fish, crustacean and mollusc species, this may be a potential cause for cross-reactivity between vertebrate and invertebrate species particularly with the highly conserved structures demonstrated for tropomyosins. Allergenicity and IgE recognition of tropomyosin has been well documented in crustacean, mollusc and fish (tilapia). A 2018 study reported the potential cross-reactivity of tropomyosin between shellfish and fish (Gonzalez-Fernandez et al., 2018). Serum from patients who have a history of adverse reactions to either shellfish or fish showed IgE-reactivity to cod and albacore tropomyosin. This highlights the need for further investigations to determine the potential of cross-reactivity across a wide range of vertebrate and invertebrate species. Triose-phosphate isomerase has been independently identified in all three groups. Aldolase A, β -enolase as well as vitellogenin have been identified in both fish and crustacean species, but further investigation is required to confirm potential IgE cross-reactivity.

5. Recombinant allergens

Fish parvalbumins and shellfish tropomyosins are the most studied seafood allergens and thus have been expressed as recombinant proteins in numerous studies. The plasmid construct is typically designed based on a published nucleotide sequence for an open reading frame, a cDNA sequence obtained from extracted total RNA or an amino acid sequence of the allergen retrieved from databases such as the NCBI GenBank and UniProt. The gene sequence is subsequently codon-optimised according to the expression host. The resulting gene sequence is usually flanked by two endonuclease (restriction enzyme) sites to facilitate cloning. An additional sequence is inserted, or often already available on the expression vector, for a hexa-histidine (His-)tag or glutathione S-transferase (GST-)tag on either the N- or C-terminus of the protein for purification by affinity chromatography (Sharp et al., 2014; Kumeta et al., 2017; Vologzhannikova et al., 2017). The pET vector system (Novagen) with a T7 or T7 lac promoter and His-tag was most commonly used for the expression of recombinant seafood allergens. The use of pET vectors is described in studies from 1999 through to 2017, suggesting that it is one of the most popular and possibly successful expression vectors. Another commonly used expression vector is the pGEX system (GE Healthcare), which incorporates a GST fusion tag. The *Escherichia coli* BL21 expression system is the dominant system to produce recombinant seafood allergens with the most

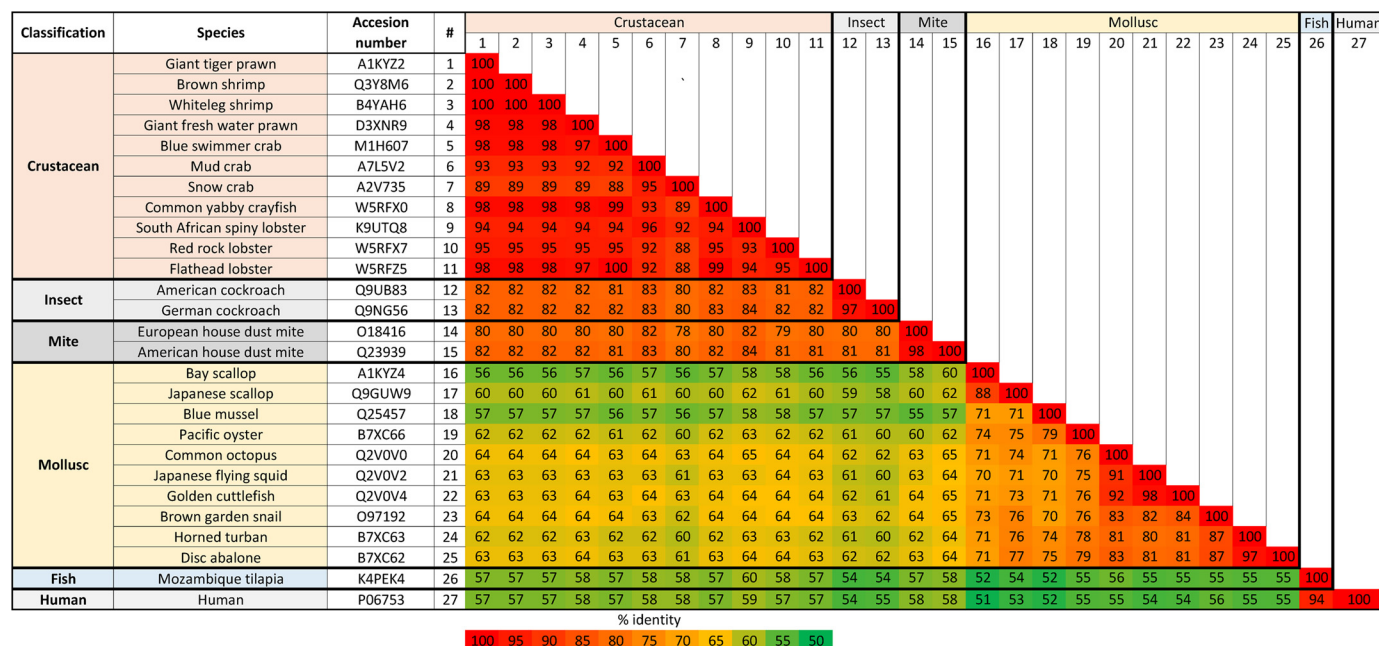


Fig. 4. Comparison of amino acid sequence identities (%) of tropomyosins. The sequence identities were calculated using Multiple Sequence Alignment in Clustal Omega (EMBL-EBI).

common subset strain being BL21(DE3). However, the users must be reminded that the proteins expressed in prokaryotic hosts do not undergo many of the post-translational modifications required for proteins originally from eukaryotic organisms to obtain their correct physiological structure and properties. Common modifications such as acetylation and phosphorylation of expressed proteins are achievable in some of the prokaryotic expression hosts (Cain et al., 2014). In the case of parvalbumin, there are no additional modifications besides the N-terminal acetylation (see Section 7.1), which negates the need for a more complex eukaryotic expression system.

5.1. Recombinant fish allergens

Successful expression and purification of recombinant parvalbumins has been demonstrated for several fish species including Atlantic cod (Van Do et al., 2003; Ma et al., 2008; Sanchez et al., 2016), Atlantic salmon (Van Do et al., 1999), barramundi (Sharp et al., 2014), carp (Swoboda et al., 2002b; Ma et al., 2008; Zuidmeer-Jonghejan et al., 2015), Pacific mackerel (Hamada et al., 2004; Kumeta et al., 2017), and wolf-herring fish (Mohammadi et al., 2017), as summarised in Table 5. Recombinant fish parvalbumins have been expressed to explore potential use in immunotherapy (Swoboda et al., 2007) and treatment (Freidl et al., 2017) of fish allergy. In addition, the recombinant parvalbumins were used in studies investigating IgE-binding and differences in secondary structures between the natural and recombinant forms of the protein (Ma et al., 2008), characterisation of isoforms of parvalbumin (Sharp et al., 2014), and determination of tertiary structure by X-ray diffraction crystallography (Cates et al., 1999) and nuclear magnetic resonance (NMR) (Kumeta et al., 2017). The introduction of mutations in the nucleotide sequence can result in the production of hypoallergenic or mutated forms of parvalbumin, which are explored in the aforementioned studies. Parvalbumin requires the presence of Ca^{2+} to obtain the correct physiological protein conformation (refer to Section 8.1 for details). The recombinant parvalbumin must be introduced to Ca^{2+} -supplemented buffer post-purification to avoid a conformation similar to that of Ca^{2+} -depleted parvalbumin. The natural parvalbumin purified from muscle tissue can however retain the bound Ca^{2+} despite undergoing the purification process (Ma et al., 2008). The IgE binding of recombinant parvalbumins

was often found to be comparable to that of the natural form (Ma et al., 2008; Sharp et al., 2014).

Recombinant tropomyosin has been expressed for Atlantic salmon (Jackman et al., 1996), however, this was used in a study unrelated to allergy. Other fish allergens such as β -enolase, aldolase A, collagen, and vitellogenin are yet to be produced as recombinant proteins (March 2018).

5.2. Recombinant crustacean allergens

Crustacean allergens have been recombinantly expressed and purified for several research purposes. The focus of recombinant crustacean allergen studies have been the discovery and characterisation of novel major allergens from previous uncharacterised species (Reese et al., 2005; Motoyama et al., 2007; Liang et al., 2008; Nakano et al., 2008; Albrecht et al., 2009; Kunimoto et al., 2009; Bauermeister et al., 2011; Abramovitch et al., 2013; Myrset et al., 2013). In addition, recombinant crustacean allergens have also been produced for IgE epitope mapping studies, which are primarily focused on the immunological characterisation of major allergens. These recombinant allergens have also been investigated for the development of novel allergy-diagnostic platforms for accurate quantification of allergen-specific IgE, as well as improving *in-vitro* diagnostics to better predict the severity of allergy to crustaceans, and to avoid risky food challenges (Albrecht et al., 2009; Johnston et al., 2014; Koeberl et al., 2014; Pascal et al., 2015). Studies investigating the effect of heat processing on major crustacean allergens, allergen detection (Kamath et al., 2014b), therapeutic research (Wai et al., 2014, 2016; Leung et al., 2017), and immune mechanisms of crustacean allergy (Leung et al., 2008; Lam et al., 2015; Mahajan et al., 2017) have also made use of recombinant allergens. Tropomyosin is the major crustacean allergen that has been widely expressed as a recombinant protein. Recombinant tropomyosins from species such as black tiger prawn (*Penaeus monodon*) (Pen m 1), Brown shrimp (*Penaeus aztecus*) (Pen a 1) and white-leg shrimp (*Litopenaeus vannamei*) (Lit v 1) have been commonly used for the above-mentioned research. However, arginine kinase, myosin light chain, sarcoplasmic calcium-binding protein, troponin C and triose-phosphate isomerase have also been produced as recombinant proteins primarily for novel allergen characterisation purposes (Ayuso et al., 2008, 2009; Bauermeister et al.,

Table 4
WHO/IUIS registered mollusc allergens. Mollusc allergens registered with the WHO/IUIS (www.allergen.org) are listed in alphabetical order of given allergen names. Note: '*' reference is not directly linked to the WHO/IUIS allergen entry. MW = molecular weight in kDa and '-' = no details available.

Mollusc species	Allergen	Protein type	MW	Genbank protein accession number of isoform		References
				1.0101	1.0102	
Pacific Oyster	<i>Crassostrea gigas</i>	Cra g 1	38	KY549366.1	AB444943.1	(Nugraha et al., 2018)*
Jade tiger abalone	<i>Haliotis laevigata</i> x <i>H. rubra</i>	Hal l 1	33	KX961689	-	-
Abalone	<i>Haliotis midae</i>	Hal m 1	49	-	-	(Lopata et al., 1997)
Brown garden snail	<i>Helix aspersa</i>	Hel as 1	36	Y14855	-	(Asturias et al., 2002)
Sydney Rock Oyster	<i>Saccostrea glomerata</i>	Sac g 1	38	MF996471	-	-
Japanese flying squid	<i>Todarodes pacificus</i>	Tod p 1	38	-	-	(Miyazawa et al., 1996)

2011; Shen et al., 2011; Pascal et al., 2015) (Table 6). Recombinant crustacean allergens have been most commonly expressed in an *E. coli* expression system, while other expression systems such as yeast (*Pichia pastoris*) have also been used (Kumjim et al., 2016).

5.3. Recombinant mollusc allergens

Recombinant tropomyosins from different mollusc species have been expressed in *E. coli* (Table 7). Tropomyosins from abalone (*Haliotis diversicolor*), scallop (*Chlamys nobilis*), and mussel (*Perna viridis*) were the first recombinantly expressed mollusc allergens reported (Chu et al., 2000). Subsequently, allergenic tropomyosins from three additional mollusc species were produced over the last 17 years. Besides tropomyosin, only allergenic paramyosin and triosephosphate isomerase from abalone (Suzuki et al., 2014) and octopus (Yang et al., 2017), respectively, were recombinantly expressed. Notably, a high salt concentration of 500 mM was needed to purify recombinant paramyosin from the crude extract. All recombinant mollusc allergens have solely been utilised for investigating their IgE reactivity.

6. Purified natural allergens

Purifying naturally occurring allergens in their native form is essential for the study of food allergy. The most commonly used method to extract soluble seafood allergens from the muscle tissue is by mechanical homogenisation in isotonic buffer (i.e. PBS and Tris-HCl). The homogenate can be left in the isotonic buffer from several hours to overnight at 4°C with gentle shaking to allow leaching of soluble proteins, which are subsequently separated from the insoluble proteins by a centrifugation step typically ranging from 5000 to 20,000 × g (Sharp et al., 2015; Kobayashi et al., 2016d; Ruethers et al., 2018). The use of protease inhibitors is often not necessary as most food allergens are resistant to proteolytic digestion (Moreno, 2007); however, it can be supplemented in the extraction buffer for a comprehensive protein characterisation (Vologzhannikova et al., 2017) and the identification of isoforms of the allergen (Brownridge et al., 2009). The muscle tissue, homogenate or the crude extract of soluble proteins can be heated or boiled for several minutes before the purification of heat-stable allergens, such as parvalbumin (Sharp et al., 2015; Kobayashi et al., 2016d) and tropomyosin (Kamath et al., 2014a). Due to their thermostability, the boiling step allows the preparation of a fraction highly enriched with these heat-stable proteins (Saptarshi et al., 2014; Vologzhannikova et al., 2017; Ruethers et al., 2018). Natural allergens are often successfully purified by chromatography. Several methods have been reported for seafood allergen purification, and the most commonly applied techniques are a combination of gel filtration (GF) (Kuehn et al., 2010; Kobayashi et al., 2016b; Freidl et al., 2017) and ion-exchange chromatography (IE) (Kuehn et al., 2010; Perez-Gordo et al., 2011). Purified allergens can be further refined by methods such as reversed-phase HPLC (Hamada et al., 2004; Guo et al., 2012). The addition of ammonium sulfate to saturate a clarified extract or fraction also allows the purification of allergen by precipitation of contaminating proteins (Bugajska-Schretter et al., 2000; Beale et al., 2009; Abdel Rahman et al., 2011; Ruethers et al., 2018).

6.1. Purified natural fish allergens

The most commonly reported fish allergen purified from muscle tissues is parvalbumin. Parvalbumin has been purified from different fish species by ammonium sulfate precipitation and/or sequential IE and GF chromatography. Some of the extraction sources include: Atlantic cod (de Jongh et al., 2011; Freidl et al., 2017), Atlantic mackerel (Kuehn et al., 2010), Atlantic salmon (Kuehn et al., 2010; Freidl et al., 2017), carp (Kuehn et al., 2010; Freidl et al., 2017), European anchovy (Beale et al., 2009), grass carp (Li et al., 2014), hake (Beale et al., 2009), herrings (Kuehn et al., 2010), Indian mackerel

(Ruethers et al., 2018), Japanese eel (Hamada et al., 2004), Pacific mackerel (Hamada et al., 2004; Guo et al., 2012; Freidl et al., 2017), trout (Kuehn et al., 2010), yellowfin tuna (Ebo et al., 2010; Kuehn et al., 2010), and other species extensively listed in Table 8. Purification of parvalbumin from carp semen has been reported (Dietrich et al., 2014); however not yet described for other fish species. Less frequently reported purified fish allergens are: tropomyosin from Mozambique tilapia (Liu et al., 2012), Atlantic salmon and several other species (Huang and Ochiai, 2005), aldolase A and β -enolase from Atlantic cod, Atlantic salmon and yellowfin tuna (Kuehn et al., 2013), and collagen from several fish species (Hamada et al., 2001, 2003). Purification of fish tropomyosin included IE and reversed-phase HPLC, while for purification of aldolase A and β -enolase a combination of IE and GF was used. Collagen or gelatin (hydrolysed collagen) can also be purified from fish skin, bone and scale (Sakaguchi et al., 2000; Koppelman et al., 2012; Pan et al., 2012; Gistelink et al., 2016; Kobayashi et al., 2016b) and a more vigorous extraction method must be applied. It requires delipidation by alkali-treatment (i.e. NaOH), and de-calcification (i.e. EDTA) steps, and finally purification of the acid-soluble collagen in acetic acid (Gistelink et al., 2016). Depending on the level of purity to be achieved, lipids are removed by butyl alcohol and pigments bleached by hydrogen peroxide (Anand et al., 2013).

6.2. Purified natural crustacean allergens

Many of the known crustacean allergens have been purified from natural sources, as listed in Table 9 (note: this list is not exhaustive). The most commonly purified allergens are tropomyosin and arginine kinase. Tropomyosin has been isolated from many shrimp and prawn species (Shiomi et al., 2008; Liu et al., 2011a; Wang et al., 2012; Gamez et al., 2015; Lasekan et al., 2017) as well as American lobster (Motoyama et al., 2007), while arginine kinase has been isolated from a few shrimp species (Yu et al., 2003; Garcia-Orozco et al., 2007; Chen et al., 2013b), mud crab (Shen et al., 2011) and horseshoe crab. MLC, SCBP and troponin have been purified from a limited number of crustacean species (Ojima et al., 1995; Chen et al., 2013a; Mita et al., 2013; Morii et al., 2013; Rohrbach et al., 2015; Zhang et al., 2015; Hu et al., 2017). These crustacean allergens have been purified not only for studies in allergy but also for studies of the molecular function of muscle (tropomyosin, MLC and troponin) and mechanisms of energy transfer by phosphagen kinases. Purification methods for crustacean allergens have made use of standard procedures such as salting out, isoelectric precipitation and column chromatography. Additionally, as tropomyosin, MLC and SCBP are heat stable, purification often involves a heating step.

Table 5

Recombinant allergenic parvalbumins in the literature. A summary of recombinant fish allergens and expression methods are listed. The WHO/IUIS name and accession number to either the nucleotide or amino acid sequence are provided where available. Note: '-' = no details available.

Fish species	Allergen	Accession number	Expression vector	Expression system	References
Atlantic cod	Gad m 1.01, Gad m 1.02	A51874 AM497927 AM497928	pET-15b; pET-17b; pET-19b	<i>E. coli</i> BL21(DE3); BL21(DE3)-RIPL; BL21Star(DE3)pLysS	(Van Do et al., 2003; Ma et al., 2008; Sanchez et al., 2016)
Atlantic salmon	Sal s 1	X97825	pET-19b	<i>E. coli</i> BL21(DE3)pLysS	(Van Do et al., 1999)
Barramundi	Lat c 1.01, Lat c 1.01	KF021278.1 KF021279.1	pPROEX HTb	<i>E. coli</i> NM522	(Sharp et al., 2014)
Common carp	Cyp c 1.01, Cyp c 1.02	AJ292211 AJ292212	pET-28b+; pET-17b	<i>E. coli</i> BL21(DE3); BL21(DE3)-RIPL	(Swoboda et al., 2002a; Ma et al., 2008; Zuidmeer-Jongejan et al., 2015)
Pacific mackerel	-	P59747	pGEX-6p-1, pGEX-6p-3	<i>E. coli</i> BL21(DE3), <i>E. coli</i> JM 109	(Hamada et al., 2004; Kumeta et al., 2017)
Wolf-herring fish	-	Primers designed based on JN190495	pET-28a	<i>E. coli</i> BL21	(Mohammadi et al., 2017)

6.3. Purified natural mollusc allergens

Tropomyosin from seven mollusc species, arginine kinase and triosephosphate isomerase from octopus and paramyosin have been purified for allergy studies using a variety of multiple step purification systems (Table 10). After ammonium sulfate precipitation, the purity was improved using chromatography methods. Only one of the respective studies mentions a yield: 33 mg of tropomyosin was purified from 44 g of muscle tissue from the brown garden snail (Asturias et al., 2002).

7. Post-translational modifications

Post-translational modifications of known seafood allergens are not very common. The few known modifications are described below.

7.1. Post-translational modifications of fish allergens

Parvalbumin, tropomyosin, β -enolase and aldolase A have been described as official WHO/IUIS-registered fish allergens. The homologous forms of all four proteins have been extensively studied as they are commonly and ubiquitously expressed within other species including human. Despite this, parvalbumin is the only fish allergen elucidated for post-translational modifications. The only described modification for fish parvalbumin is the alpha-N-terminal acetylation (Lee et al., 2009; Dietrich et al., 2014), and occurs on both the α - and β -parvalbumins (Permyakov et al., 2012). Mass spectrometry (MS) is often utilised in analysing the protein moieties. The addition of an acetyl group on the N-terminal region on the majority of the purified natural Gad m 1 was observed by ESI-QTOF intact MS analysis (Ma et al., 2008). Furthermore, the N-terminal alanine was found to undergo the modification, confirmed by LC-MS/MS using tryptic-digested peptides. The α -parvalbumin from human and β -parvalbumin from rat were subjected to ESI-MS and determined to have no additional modification (Fohr et al., 1993).

The presence of post-translational modifications on several fish tropomyosins was suggested by MALDI-TOF MS analysis based on the observed mass differences to the theoretical mass (Huang and Ochiai, 2005). There is no information on the exact modifications that cause these mass differences of fish tropomyosins; however, it is speculated that they undergo modifications other than N-terminal acetylation commonly found on tropomyosin from other species.

α - and β -enolases and aldolase A from non-fish species undergo acetylation, phosphorylation and methylation (Freemont et al., 1988; Merkulova et al., 1997). The only study describing cod, salmon and

Table 6

Recombinant WHO/IUIS-registered and reported crustacean allergens in the literature. A summary of recombinant crustacean allergens and expression methods are listed. The WHO/IUIS name and accession number to either the nucleotide or amino acid sequence are provided where available. Note: ‘-’ = no details available.

Protein	Crustacean species	Allergen	Accession number	Expression vector	Expression system	References
Tropomyosin	Kuruma prawn	–	BAF47263	pGEM-T	<i>E. coli</i> BL21	(Kunimoto et al., 2009)
	Northern shrimp	Pan b 1	FR728681	pET19b	<i>E. coli</i> Rosetta2 (DE3)	(Myrset et al., 2013)
	Krill	–	AB289604	pCold IV	<i>E. coli</i>	(Nakano et al., 2008)
	Chinese mitten crab	–	EF471314	pGEX-4	<i>E. coli</i> JM109	(Liang et al., 2008)
	Brown shrimp	Pen a 1	DQ151457	pET101	<i>E. coli</i> BL21 (DE3)	(Reese et al., 2005)
	North sea shrimp	Cra c 1	FJ457621	pET23b	<i>E. coli</i> BL21	(Bauermeister et al., 2011)
	Blue swimmer crab	Por p 1	JX874982	pProEXHT-B	<i>E. coli</i> NM522	(Abramovitch et al., 2013)
	King prawn	Mel l 1	JX171685	pProEXHT-B	<i>E. coli</i> BL21 (DE3) RIPL	(Koeberl et al., 2014)
	Black tiger prawn	Pen m 1	HM486525	pRSET-A	<i>E. coli</i> BL21 (DE3)	(Kamath et al., 2014a)
	Giant freshwater shrimp	Mac r 1	GU369816	pPICZαA	<i>Yeast Pichia strain</i> KM71	(Kumjim et al., 2016)
Arginine kinase	Flowery crab	Cha f 1	AF061783	pGEX 1	<i>E. coli</i>	(Leung et al., 1998b)
	North sea shrimp	Cra c 2	FJ457622	pET23b	<i>E. coli</i> BL21	(Bauermeister et al., 2011)
	Mud crab	–	GQ851626	pGEX-4T-3	<i>E. coli</i> BL21	(Shen et al., 2011)
	White-leg shrimp	Lit v 2	ABY57915	pGEX-4T-2	<i>E. coli</i> BL21(DE3) codon plus	(Yao et al., 2009)
Myosin light chain	Greasyback shrimp	–	EU497674	pET28a	<i>E. coli</i> Rosetta	(Wang et al., 2009)
	White-leg shrimp	Lit v 3	EU449515	pET24b	<i>E. coli</i> BL21	(Ayuso et al., 2008)
Sarcoplasmic calcium-binding protein	North sea shrimp	Cra c 5	FJ462739	pET23b	<i>E. coli</i> BL21	(Bauermeister et al., 2011)
	White-leg shrimp	Lit v 4	FJ184279	pET24b	<i>E. coli</i> BL21	(Ayuso et al., 2009)
	Black tiger prawn	Pen m 4	ADV17343	pFN6A (HQ) Flexi	<i>E. coli</i> KRX	(Mita et al., 2013)
	North sea shrimp	Cra c 4	FJ462737	pET23b	<i>E. coli</i> BL21	(Bauermeister et al., 2011)
	Red swamp crayfish	–	AEG79568.1, AEG79569.1, AEG79567.1	pET21b	<i>E. coli</i> BL21(DE3)	(Rohrbach et al., 2015)
Troponin C	Mud crab	–	AFJ80778	pET22b	<i>E. coli</i> Rosetta	(Hu et al., 2017)
	Black tiger prawn	Pen m 6	ADV17344	pET32a	<i>E. coli</i> BL21(DE3)	(Kalyanasundaram and Santiago, 2015)
Triosephosphate isomerase	North Sea shrimp	Cra c 6	FJ462740	pET23b	<i>E. coli</i> BL21	(Bauermeister et al., 2011)
	North Sea shrimp	Cra c 8	FJ462738	pET23b	<i>E. coli</i> BL21	(Bauermeister et al., 2011)
Hemocyanin	White-leg shrimp	–	AFT92034.1	pJetExpress404	<i>E. coli</i> BL21(DE3)	(Lopez-Zavala et al., 2016)
	Chinese mitten crab	–	AEG64817	pET28a	<i>E. coli</i> Rosetta (DE3)	(Zhang et al., 2016)

tuna β -enolases and aldolase A as fish allergens analysed these protein's moieties and reported no modifications; however, supplementary data was not provided (Kuehn et al., 2013).

7.2. Post-translational modifications of crustacean and mollusc allergens

Little is known about the post-translational modification of most crustacean and mollusc allergens. The most extensively studied allergen in this area is hemocyanin. Hemocyanin from white-leg shrimp was shown to be heavily glycosylated and this may be variant specific. It is

thought this glycosylation has a role in the immune response for the agglutination and clearance of bacteria (Zhang et al., 2017). Furthermore, cleavage of the C-terminal end of hemocyanin releases a fragment that has antimicrobial activity (Wen et al., 2016). A *Vibrio parahaemolyticus* resistant white-leg shrimp was found to have a 18.4 kDa truncated hemocyanin in its serum. A small antibacterial peptide of hemocyanin from freshwater crayfish (*Pacifastacus leniusculus*), hemocyanin, has also been discovered (Lee et al., 2003). In mollusc hemocyanin (e.g. some snails) a variety of N-glycans have been detected as well as the methylation of hexoses, which is a novel modification

Table 7

Recombinant WHO/IUIS-registered and reported mollusc allergens in the literature. A summary of recombinant crustacean allergens and expression methods are listed. The WHO/IUIS name and accession number to either the nucleotide or amino acid sequence are provided where available. Note: ‘-’ = no details available.

Protein	Mollusc species	Allergen	Accession number	Expression vector	Expression system	References
Tropomyosin	Abalone	–	X75218.1	pGEX	<i>E. coli</i> DH5α	(Chu et al., 2000)
	Scallop	–	AB021681.1	proEX HT	<i>E. coli</i> XL1-Blue	
	Mussel	–	AB000907.1			
	Pacific oyster	Cra g 1	KY549366.1, AB444943.1	pGEX4T 1-3	<i>E. coli</i> XL1-Blue	(Leung and Chu, 2001)
	Snail	Hel as 1	Y14855	pKN172	<i>E. coli</i> JM109 (DE3)	(Asturias et al., 2002)
	Chinese razor clam	–	EU082209	pET28a	<i>E. coli</i> BL21(DE3)	(Song et al., 2009)
Paramyosin	Abalone	–	AB571843	pFN6A (HQ) Flexi	<i>E. coli</i>	(Suzuki et al., 2014)
Triosephosphate isomerase	Octopus	–	KT007222	pET-28a	<i>E. coli</i> BL21(DE3)	(Yang et al., 2017)

(Staudacher et al., 2009).

Myosin light chains are modified by phosphorylation and glycosylation. As part of the physiological function of myosins, the regulatory light chains need to be phosphorylated by myosin light-chain kinase. This is part of the muscle contractile action. MLC1 from red swamp crayfish has been shown to be a glycoprotein that contains 4.3% carbohydrates (Zhang et al., 2015). SCBP from red swamp crayfish was also identified as a glycoprotein, containing 4.7% carbohydrates (Chen et al., 2013b). No other modifications of MLC and SCBP have been reported.

Crustacean and mollusc tropomyosin have not been explored in detail for post-translational modifications; however, modifications in tropomyosins of other species (e.g. mammals and yeast) are described such as acetylation, phosphorylation, S-nitrosylation and disulfide bonding (Schevzov et al., 2011). One crustacean example is tropomyosin from American lobster. This species contains fast and slow tropomyosin isoforms and it is suggested that they are both generated as splicing variants of the same gene. Furthermore, N-terminal acetylation and another post-translational modifications are indicated due to differences in mass obtained by mass spectrometry (Mykles et al., 1998).

8. Available structures of seafood allergens

The determination of the tertiary structure of allergens leads to an understanding of a protein's behaviour but its foremost benefit is in the elucidation of potential IgE-binding regions. Several structures of seafood allergens have been characterised and are available through the Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) (www.rcsb.org). The following sections will introduce and discuss in detail, the features of published seafood allergen structures.

8.1. Available structures of fish allergens

Of the fish allergens, parvalbumin is the only protein studied for its tertiary structure. Parvalbumin consists of six α -helices (A–F) and short segments of antiparallel β -sheet, organised by the N-terminal AB domain and the C-terminal CD-EF domain. The metal ion-binding sites reside within the CD and EF loops, which are flanked by the corresponding helical elements. There are a total of 20 parvalbumin structures from six bony-fish species deposited in PDB (March 2018), which are summarised in Table 11. Most parvalbumin structures were solved by the X-ray power diffraction of crystalline parvalbumin (crystallography) and their data were often published in the 1990s, with the highest atomic resolution of 0.91 Å (Declercq et al., 1999). More recently published structures were determined by NMR and in particular,

solution NMR could be used to determine epitopes important for IgE-binding (Kumeta et al., 2017). Although their allergenicity is yet to be determined, the only structure available for cartilaginous fish parvalbumin is α -parvalbumin (PDB: 5PAL) from Leopard shark (*Triakis semifasciata*), which became available in 1993. In comparison to bony fish β -parvalbumins, α -parvalbumin differed in the C-terminal organisation due to an elongation induced by one additional amino acid residue despite the main structural features being conserved (Roquet et al., 1992).

Many of the purified natural and recombinant parvalbumins were found to form dimers and oligomers (van der Ventel et al., 2011; Sharp et al., 2014, 2015; Zuidmeer-Jonghejan et al., 2015; Vologzhannikova et al., 2017). Four structures of common carp parvalbumin were resolved as homodimers (Table 11); however, none of the original studies further investigated the occurrence of dimerisation of parvalbumin. The transient dimers of allergens are speculated to have significantly higher allergenicity compared to the monomeric form (Rouvinen et al., 2010) due to feasible cross-linking between multiple IgEs bound on the surface of mast cells via Fc-epsilon receptors (FcεR), activating mast cell degranulation (Shakib et al., 2008). This has been observed for cow's milk allergen, Bos d 5 (Rouvinen et al., 2010), and more recently for Indian mackerel parvalbumin, Ras k 1. (Ruethers et al., 2018).

It is important to note that many of these parvalbumin structures were determined while bound to metal ions, particularly calcium or magnesium, regardless of the phylogenetic lineages. Parvalbumin is a calcium-binding protein and it cannot achieve its physiological conformation without the presence of divalent ions bound on the EF-hand domains (CD and EF sites) of the protein (Fig. 5A) (Declercq et al., 1999). It also affects the protein stability by significantly reducing the melting temperature and the reversibility of folding. Ca^{2+} -depleted parvalbumin prepared by EGTA-treatment is more prone to denaturation at a temperature as low as 30°C and completely loses the ability to refold after heat treatment (Bugajska-Schretter et al., 2000; Ma et al., 2008). Several studies using rat and chicken α - and β -parvalbumins elucidated the conformational change influenced by the removal of Ca^{2+} . The most prominent conformational alteration was observed on the CD-loop binding site of Ca^{2+} -depleted β -parvalbumin (Henzl and Tanner, 2007; Henzl et al., 2011), while a less extensive change was induced in β -parvalbumin.

The additional feature to be noted on parvalbumin structures is the presence of the acetyl group. One of the post-translational modifications known for parvalbumin is alpha-N-acetylation (N-terminal acetylation). The absence of N-terminal acetylation can drastically change the protein folding, degradability and increases the propensity of oligomerisation (Permyakov et al., 2012), and therefore almost all parvalbumin structures determined were acetylated.

Table 8

Natural WHO/IUIS-registered and reported fish allergens in the literature. The purified natural fish allergens from respective sources are summarised i with purification methods used. The WHO/IUIS name and accession number to either the nucleotide or amino acid sequence are provided where available. Note: ASP = ammonium sulfate precipitation, GF = gel filtration chromatography, HIC = hydrophobic interaction chromatography, IE = ion-exchange chromatography, P = precipitation, RP-HPLC = reversed-phase high-performance liquid chromatography and ‘-’ = no details available. * gelatin is hydrolysed collagen.

Protein	Fish species	Allergen	Purification method	References
Parvalbumin	Alaska pollack	–	IE, GF, RP-HPLC	(de Jongh et al., 2011; Perez-Gordo et al., 2011; Freidl et al., 2017)
	Atlantic cod	Gad m 1	IE, GF; ASP, IE; IE	(Kuehn et al., 2010)
	Atlantic salmon	Sal s 1	IE, GF; IE	(Kuehn et al., 2010; Perez-Gordo et al., 2011; Kuehn et al., 2013)
	Bigeye tuna	–	GF, RP-HPLC	(Hamada et al., 2001, 2004)
	Common carp	Cyp c 1	ASP, IE	(Bugajska-Schretter et al., 2000; Kuehn et al., 2010; Freidl et al., 2017)
	Crimson sea bream	–	GF, RP-HPLC	(Guo et al., 2012)
	European anchovy	–	ASP, IE	(Beale et al., 2009)
	Grass carp	–	GF, HPLC	(Li et al., 2014)
	Hake	–	ASP, IE	(Beale et al., 2009)
	Herring	Clu h 1	IE, GF	(Kuehn et al., 2010)
	Horse mackerel	–	GF, RP-HPLC	(Hamada et al., 2004; Guo et al., 2012)
	Indian mackerel	Ras k 1	ASP	(Ruethers et al., 2018)
	Pacific mackerel	–	GF, RP-HPLC	(Hamada et al., 2004; Guo et al., 2012; Kobayashi et al., 2016b)
	Pangasius	–	IE, GF	(Ebo et al., 2010)
	Ocean perch	Seb m 1	IE, GF	(Kuehn et al., 2010)
	Rainbow trout	Onc m 1	IE, GF	(Kuehn et al., 2010)
	Whiff	Lep w 1	IE	(Perez-Gordo et al., 2011)
	Wolf-herring	–	IE	(Mohammadi et al., 2017)
	Yellowfin tuna	Thu a 1	IE, GF	(Ebo et al., 2010; Kuehn et al., 2010)
	Yellowtail	–	ASP, IE	(Beale et al., 2009)
β-enolase	Atlantic cod	Gad m 2	IE, GF	(Kuehn et al., 2013)
	Atlantic salmon	Sal s 2	IE, GF	
	Yellowfin tuna	Thu a 2	IE, GF	
Aldolase A	Atlantic cod	Gad m 3	IE, GF	
	Atlantic salmon	Sal s 3	IE, GF	
	Yellowfin tuna	Thu a 3	IE, GF	
Tropomyosin	Mozambique tilapia	Ore m 4	IE, RP-HPLC	(Liu et al., 2013)
Collagen	Bigeye tuna	–	Alkaline, Acid	(Hamada et al., 2001)
	Pacific mackerel	–	Alkaline, Acid	(Kobayashi et al., 2016b)
	Redbelly tilapia	–	Alkaline, butyl alcohol, Acid	(Pan et al., 2012)
Gelatin*	Tuna	–	Boil, Acid, Salt P	(Sakaguchi et al., 2000)
	Mackerel	–	Boil, Acid, Salt P	
	Salmon	–	Boil, Acid, Salt P	
	Saurel	–	Boil, Acid, Salt P	

The structures of other fish allergens are yet to be determined. The crystal structures of aldolase, β-enolase and collagen (potential allergen) from other species are available; hence those with the highest

sequence identity to available fish allergen sequences are depicted as representative proteins to show the morphology in Fig. 5. The structure of tropomyosin is well conserved across phylogenetically distant

Table 9

Natural WHO/IUIS-registered and reported crustacean allergens in the literature. The purified natural crustacean allergens from respective sources are summarised with purification methods used. The WHO/IUIS name and accession number to either the nucleotide or amino acid sequence are provided where available. Note: ASP = ammonium sulfate precipitation, GF = gel filtration chromatography, IE = ion-exchange chromatography, IEP = isoelectric precipitation, RP-HPLC = reversed-phase high-performance liquid chromatography and ‘-’ = no details available.

Protein	Crustacean species	Allergen	Purification method	References
Tropomyosin	Black tiger prawn	Pen m 1	ASP, IEP, RP-HPLC; IEP, ASP	(Shiomi et al., 2008; Wang et al., 2012; Lasekan et al., 2017)
	Kuruma prawn	–	IEP, ASP, IE	(Usui et al., 2013)
	White-leg shrimp	Lit v 1	IEP, ASP	(Liu et al., 2011a)
	Razor mud shrimp	–	IEP, ASP	(Gamez et al., 2015)
	American lobster	Hom a 1	IEP, ASP, RP-HPLC	(Motoyama et al., 2007)
Arginine kinase	Red swamp crayfish	–	ASP, IE	(Chen et al., 2013b)
	Mud crab	–	ASP, IE	(Shen et al., 2011)
	Black tiger prawn	Pen m 2	ASP, IE	(Yu et al., 2003)
	Craw fish	–	ASP, IE	(Yu et al., 2003)
	White-leg shrimp	Lit v 2	ASP, GF, IE	(Garcia-Orozco et al., 2007)
Myosin light chain	Red swamp crayfish	–	IEP, ASP, IE, GF	(Zhang et al., 2015)
Sarcoplasmic calcium-binding protein	Red swamp crayfish	–	ASP, IE, GF; GF	(Chen et al., 2013a; Rohrback et al., 2015)
	Black tiger prawn	Pen m 4	ASP, RP-HPLC	(Mita et al., 2013; Morii et al., 2013)
	Mud crab	–	ASP, IE, GF	(Hu et al., 2017)
Troponin	Kuruma prawn	–	ASP, IEP, ASP, IE	(Ojima et al., 1995)

species; hence, pig tropomyosin is depicted in Fig. 6A.

8.2. Available structures of shellfish allergens

Structural data is available for three of the shellfish allergens, arginine kinase, triosephosphate isomerase and hemocyanin, all resolved by X-ray crystallography (summarised in Table 12; images in Fig. 6). Arginine kinase structures are available from horseshoe crab and white-leg shrimp (Fig. 6B) and depict structures in the substrate-free open

state (Niu et al., 2011; Lopez-Zavala et al., 2012) and in complex with ligands (closed state), including structures of the transition state. (Lopez-Zavala et al., 2013). These different structures have helped in elucidating the conformational changes that occur to allow the transfer of phosphates. Arginine kinase is mainly α -helical and contains two domains; a small N-terminal domain and a larger C-terminal domain, which both close upon substrate binding (substrate induced fit) (Yousef et al., 2003; Niu et al., 2011; Lopez-Zavala et al., 2012). An Mg^{2+} ion is bound adjacent to the active site to facilitate the binding of ATP.

Table 10

Natural WHO/IUIS-registered and reported mollusc allergens in the literature. The purified natural crustacean allergens from respective sources are summarised with purification methods used. The WHO/IUIS name and accession number to either the nucleotide or amino acid sequence are provided where available. Note: AP = acetone precipitation, ASP = ammonium sulfate precipitation, GF = gel filtration chromatography, HIC = hydrophobic interaction chromatography, IE = ion-exchange chromatography, IEP = isoelectric precipitation, P = precipitation, RP-HPLC = reversed-phase high-performance liquid chromatography, UF = ultrafiltration and ‘-’ = no details available.

Protein	Mollusc species	Allergen	Purification method	References
Tropomyosin	Squid	Tod p 1	ASP, IE, GF	(Miyazawa et al., 1996; Nakamura et al., 2006; Jin et al., 2015)
	Pacific oyster	Cra g 1	GF, IE, RP-HPLC	(Ishikawa et al., 1997; Ishikawa et al., 1998b)
	Turbo cornutus	–	GF, IE, RP-HPLC	(Ishikawa et al., 1998a)
	Octopus	–	IE, RP-HPLC	(Ishikawa et al., 2001)
	Scallop	–	ASP, IE, UF	(Nakamura et al., 2005)
	Japanese flying squid	–	ASP, IEP, RP-HPLC	(Emoto et al., 2009)
	Snail	Hel as 1	AP, IEP, IE	(Asturias et al., 2002)
Arginine kinase	Octopus	–	ASP, IE, GF	(Chen et al., 2013b)
Paramyosin	Abalone	–	ASP, IE	(Suzuki et al., 2011, 2014)
Triosephosphate isomerase	Octopus	–	ASP, IE, GF	(Yang et al., 2017)

Table 11

Available structures of bony fish parvalbumins. Crystal- or NMR-defined structures of bony-fish parvalbumins currently available (March 2018) in the Protein Data Bank (PDB - www.rcsb.org) are summarised. Information is given for the structural resolving method, resolution and special features for each of the structures. Note: Ca^{2+} = calcium, Cd^{2+} = cadmium, Mg^{2+} = magnesium, NH_4^+ = ammonium, Mn^{2+} = manganese, calc = calculation, sub = submitted and '-' = no details available.

Fish species	Allergen	PDB accession	Resolving method	Resolution	Features to note	Year of release	References
Atlantic cod	Gad m 1	2MBX	Solution NMR	200 conformers calc. 20 conformers sub.	Ca^{2+} -bound	2014	(Moraes et al., 2014)
Common carp	Cyp c 1	1B8C	X-ray diffraction	2 Å	Mutant, homodimer, Mg^{2+} -bound	1999	(Cates et al., 1999)
		1B8L		1.7 Å	Triple mutant		
		1B8R		1.9 Å	Recombinant (<i>E. coli</i>), Ca^{2+} -bound		
		1B9A		2.0 Å	Mutant, Recombinant (<i>E. coli</i>), Ca^{2+} -bound		
		4CPV	Solution NMR	1.5 Å	Homodimer	1990	(Kumar et al., 1990)
		1CDP		1.6 Å	Homodimer, Cd^{2+} -bound		
		5CPV		1.6 Å	Homodimer, Ca^{2+} -bound		
Northern pike	-	1PAL	X-ray diffraction	1.65 Å	Ca^{2+} -bound, NH_4^+ -bound	1990	(Declercq et al., 1991)
		2PAL		1.8 Å	Mn^{2+} -bound		
		3PAL		2.4 Å	Ca^{2+} -bound, Mg^{2+} -bound		
		4PAL		1.8 Å	Ca^{2+} -bound, Mg^{2+} -bound		
	-	2PAS	Solution NMR	9 conformers sub.	Ca^{2+} -bound	1994	-
		3PAT		1 conformer sub.	Ca^{2+} -bound		
	-	1PVA	X-ray diffraction	1.65 Å	α -parvalbumin, Ca^{2+} -bound	1995	-
		1PVB		1.7 Å	β -parvalbumin, Ca^{2+} -bound, NH_4^+ -bound		
		2PVB		0.91 Å	Ca^{2+} -bound, NH_4^+ -bound, formic acid-bound	1998	(Declercq et al., 1999)
Pacific mackerel	-	5XND	Solution NMR	100 conformers calc. 20 conformers sub.	Recombinant (<i>E. coli</i>), Ca^{2+} -bound	2017	(Kumeta et al., 2017)
Silver hake	-	1BU3	X-ray diffraction	1.65 Å	Ca^{2+} -bound	1998	(Richardson et al., 2000)
Whiting	-	1A75	X-ray diffraction	1.9 Å	Ca^{2+} -bound	1998	-

The available structure for triosephosphate isomerase is from white-leg shrimp (Fig. 6F). The enzyme is a dimer with the catalytic site situated at the interface of the dimers. There are four catalytic residues and all are contained on one of the subunits. It has an extremely fast reaction mechanism and requires no additional cofactors. The enzyme is referred to as 'evolutionarily perfect' as its rate is only limited by substrate diffusion (Wierenga et al., 2010; Lopez-Zavala et al., 2016).

The hemocyanin structure has been resolved from Californian spiny lobster (*Panulirus interruptus*) (Volbeda and Hol, 1989) (Fig. 6C). It is a hexamer that is formed by three tight dimers coming together. The centre of the hexamer is lined with residues that are conserved among arthropods. There are two copper binding sites at the centre that bind one oxygen.

The other shellfish allergens do not have structures available so in lieu of this we can look at the structures from other species to get an idea of what they might look like. Species were chosen based on the highest sequence identity from available data on the PDB. Tropomyosin (Fig. 6A, wild boar, *Sus scrofa*, PDB: 1C1G), an actin-binding protein, forms a stable coiled-coil dimer due to a combination of the hydrophobic and ionic interactions of tropomyosins' unique amino acid

pattern. This unbroken pattern is a heptad repeat and follows a similar pattern to: H-P-P-H-C-P-C, where H is hydrophobic, P is polar and C is charged. According to the structure from the wild boar, tropomyosin has a length of 420 Å, appropriate for it to span seven actin monomers (Whitby and Phillips, 2000).

Other proteins shown are MLC from blood fluke (Fig. 6C, *Schistosoma mansoni*, PDB: 3JAX (Sulbaran et al., 2015)), SCBP from lancelet (Fig. 6D, *Amphioxus*, PDB: 2SAS (Cook et al., 1993)) and troponin C from giant water bug (Fig. 6E, *Lethocerus indicus*, PDB: 2JNF (De Nicola et al., 2007)).

9. Component-resolved diagnostics for seafood allergy

The wide diversity of seafood poses a challenge for clinicians to identify the exact species and allergens involved in allergic reactions. As commercially available diagnostic methods do not reflect this diversity, avoidance of all seafood is often recommended (Lopata and Kamath, 2012). Current diagnosis of seafood allergy is based on clinical history, sensitisation tests such as allergen-specific IgE quantification, and SPT, as well as oral challenge tests if required. The SPT is usually

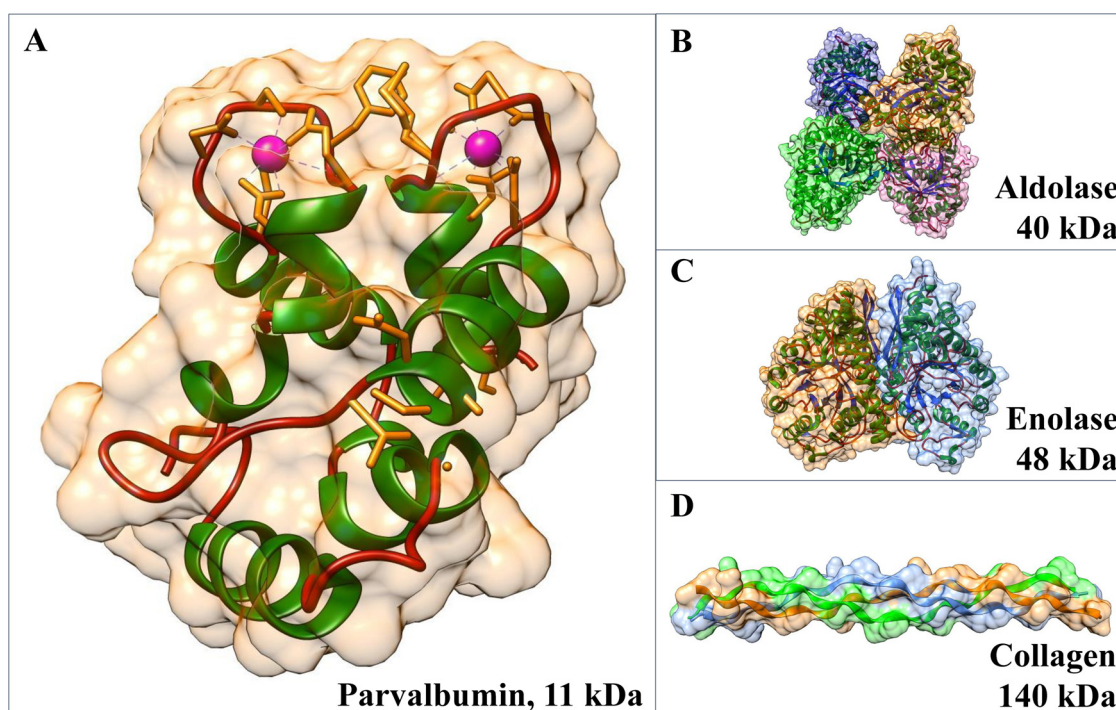


Fig. 5. Three-dimensional structural models of fish parvalbumin and representative proteins from non-fish species. Crystal structures of A) Parvalbumin from Northern pike (*Esox lucius*) (PDB: 2PVB) with two calcium ions (magenta) bound on the EF-hand domains, B) Fructose-1,6-(bis)phosphate aldolase (PDB: 1XFB) homotetramer from human, C) β -enolase (PDB: 2XSX) homodimer from human, and D) Collagen-like peptides (PDB: 1CGD) shown in a triple helix conformation. The crystal structure of human brain fructose 1,6-(bis)phosphate Aldolase (C isozyme) (PDB: 1XFB) (Fig. 5B) was prepared based on a 56% identity match to Atlantic salmon fructose-bisphosphate aldolase A (UniProt: B5DGM7 ALDOA_SALSA), and human β -enolase (PDB: 2XSX) (Fig. 5C) was prepared based on an 85% identity match to Atlantic salmon β -enolase (UniProt: B5DGQ7 ENOB_SALSA). The homology search was conducted with Swissmodel (Arnold et al., 2006). The crystal structure of synthetic hydrated collagen peptides (PDB: 1CGD) (Fig. 5D) was chosen and prepared as it is the best representative exhibiting the unique triple helix of heterologous collagen chains. The α -helices are shown in green, β -sheets in blue and the loops in red. The protein surface is indicated in transparent colours, with each colour representing a monomer of the structure. The structures were built using UCSF Chimera 1.12 (Pettersen et al., 2004) and are not to scale (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 12

Available structures of crustacean allergens. Resolved crustacean allergen structures currently available (March 2018) in the Protein Data Bank (PDB - www.rcsb.org) are summarised. Information is given for the structural resolving method, resolution and special features for each of the structures. Note: ADP-Mg = adenosine-5'-diphosphate and magnesium ion, 2-PG = 2-phosphoglyceric acid, NO_3^- = nitrate ion.

Allergen	Crustacean species	PDB accession	Resolution	Features to note	Year of release	References
Arginine kinase	White-leg shrimp	4BG4	1.6 Å	Ternary transition state analogue complex with arginine, ADP-Mg, NO_3^-	2013	(Lopez-Zavala et al., 2013)
		4BHL	1.9 Å	Binary complex with arginine		
		4AM1	1.25 Å	Unligated, monomeric	2012	(Lopez-Zavala et al., 2012)
Arginine kinase	Horseshoe crab	5J99, 5J9A	1.7 Å, 2.0 Å	Ambient temperature transition state	2016	(Godsey et al., 2016)
		4GVY, 4GVZ, 4GW0, 4GW2	2.1 Å, 3.0 Å, 2.5 Å, 2.2 Å	In complex with ADP, NO_3^- and various phosphagen analogs	2012	(Clark et al., 2012)
		3M10	1.7 Å	Substrate-free	2010	(Niu et al., 2011)
		1SD0	2.3 Å	Active site mutant	2004	(Gattis et al., 2004)
		1RL9	1.45 Å	Creatine-ADP ternary complex	2004	(Azzi et al., 2004)
		1P50, 1P52	2.8 Å, 1.9 Å	Transition state mutant	2003	(Pruett et al., 2003)
		1M15	1.2 Å	Transition state structure	2002	(Yousef et al., 2002)
		1BG0	1.9 Å	Transition state structure	1998	(Zhou et al., 1998)
Triosephosphate isomerase	White-leg shrimp	5EYW	1.7 Å	Homodimer, complexed with 2-PG inhibitor	2016	(Lopez-Zavala et al., 2016)

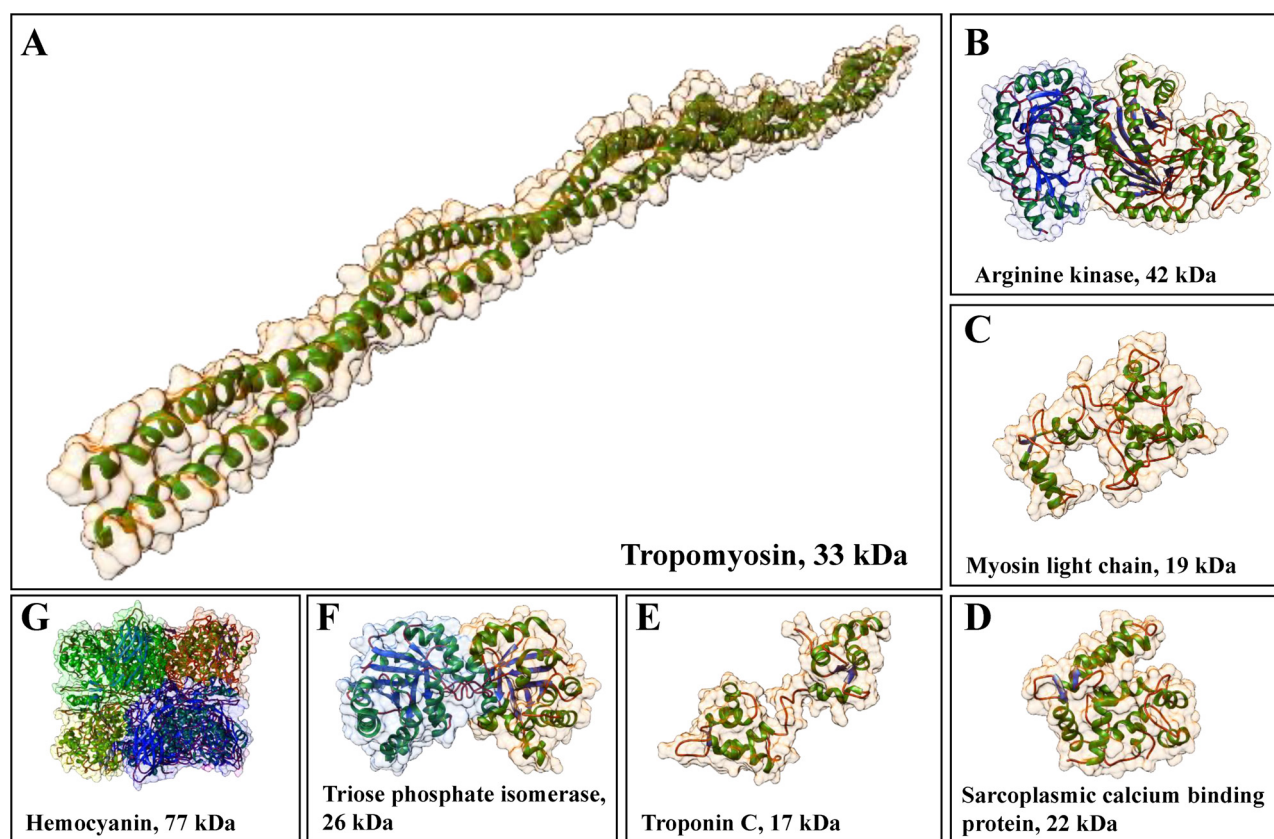


Fig. 6. Three-dimensional structural models based on existing crystal structures (RSCB Protein Data Bank) for major and minor shellfish allergens. The depicted models are based on crystal structures with the highest homology to prawn allergens (WHO/IUIS) predicted using the homology search function in Swissmodel (Arnold et al., 2006). The structures are shown in clockwise order following numeration of respective allergens by WHO/IUIS: **A**) Tropomyosin (PDB: 1C1G) dimer from pig, **B**) Arginine kinase (PDB: 4BG4) dimer from prawn, **C**) Myosin light chain (PDB: 3JAX) from blood fluke, **D**) Sarcoplasmic calcium binding protein (PDB: 2SAS) from lancelet, **E**) Troponin C (PDB: 2JNF) from waterbug, **F**) Triose phosphate isomerase (PDB: 5EYW) dimer from prawn, and **G**) Hemocyanin (PDB: 1HCY) hexamer from spiny lobster. The alpha-helices are shown in green, beta-sheets in blue and the loops in red. The protein surface is indicated in transparent colours, with each colour representing monomer of the structure. The structures were built using UCSF Chimera 1.12 (Pettersen et al., 2004) and are not to scale (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

performed with commercial preparations arriving from whole protein extracts with unknown protein composition, available from many different, often regional, manufacturers. In the case of patients with severe allergic reactions, *in vitro* IgE tests are preferred over SPT or oral food challenges to minimise the risk of severe allergic reactions.

Currently, *in vitro* IgE tests involve the use of whole natural extracts prepared from the offending source. The advantages of this approach are the ease of testing for sensitisation and the availability of commercial tests against a wide variety of seafood species on available platforms; for example, the ImmunoCAP® (Thermo Scientific), OPTIGEN®/CLA® (Hitachi Chemical Diagnostics), and Immulite® (Siemens) (Table 13). However, there are a few drawbacks. The use of natural extracts may not allow standardisation of allergen concentrations, and quantification of specific-IgE to less abundant allergens may not be accurate, leading to false-negative results. Current research is increasingly focused on the use of purified allergens for component-resolved diagnosis to overcome these drawbacks. The use of single allergens may improve the test sensitivity and specificity (markers of primary sensitisation and indicators of cross-reactivity) (Lopata et al., 2016). Developing component-resolved diagnosis for seafood allergy may require knowledge on the natural abundance of the allergens, its location in the organism, as well as its cross-reactive potential with other related allergen sources. Table 13 provides an overview of allergen sources and components available on the most common platforms for *in vitro* diagnostics. Notably, most of the species available for diagnostics are native to the Northern hemisphere. Species from the Southern Hemisphere seem to contain considerably different allergens

and isoforms (Liang et al., 2017; Ruethers et al., 2018), posing a challenge for reliable diagnostics.

The commercially available component-resolved diagnostics ImmunoCAP® platforms allow the determination of the level of specific-IgE to recombinantly expressed parvalbumin from carp (rCyp c 1) and cod (rGad c 1). However, this does not represent the wide diversity of allergenic β -parvalbumins elaborated in Section 4.1. Cyp c 1 and Gad c 1 share a sequence identity of only 59–90 and 46–68%, respectively, with other WHO/IUIS-registered fish parvalbumins.

Due to the high abundance of tropomyosin in edible crustaceans and molluscs, specific-IgE to tropomyosin is easily detected with high sensitivity. rPen a 1 from brown shrimp (*P. aztecus*) on the ImmunoCAP® and rPen m 1 from black tiger prawn (*P. monodon*) on the Immulite® systems are commercially available for testing. Due to the highly cross-reactive nature of tropomyosin among crustaceans, molluscs, dust mites, and other invertebrate species, there might be an issue of false-positive detection. Testing against purified tropomyosin from diverse species may provide more information on the primary sensitiser and cross-reactivity to other seafood sources. Several studies have demonstrated the use of specific-IgE to tropomyosin to predict clinical reactivity to shrimps in preference to shrimp SPT and/or specific-IgE to whole shrimp extract (Yang et al., 2010; Gamez et al., 2011). In addition to tropomyosin (Pen m 1), other shrimp allergens such as arginine kinase (Pen m 2) and SCBP (Pen m 4) are available on the microarray platform called ImmunoCAP® ISAC (Thermo Scientific). Interestingly, a recent study has demonstrated the use of specific-IgE to tropomyosin and SCBP in predicting clinical reactivity to shrimps (Pascal et al.,

Table 13

Allergens commercially available for *in vitro* tests in March 2018. Allergens available for component-resolved diagnostics are either recombinantly expressed (r) or purified from natural sources (n). No mollusc allergens are available for component-resolved diagnostics. The allergens available for Siemens Immulite® have been retrieved from the 3gAllergy™ catalogue for liquid allergens. Note: PV = parvalbumin, TM = tropomyosin, AK = arginine kinase and SCBP = sarcoplasmic-binding protein.

Allergen sources available for seafood allergy diagnostics				
Platform	Fish		Crustacean	Mollusc
Phadia, ImmunoCAP® Rapid	28 species		5 species	9 species
Siemens Immulite®	18 species		3 species	8 species
Hitachi OPTIGEN®/CLA®	3 species		2 species	1 species
Seafood allergens available for component-resolved diagnostics				
Platform	Fish		Crustacean	
	Allergen	Species	Allergen	Species
Phadia ImmunoCAP® Rapid	rCyp c 1 (PV)	Carp (<i>C. carpio</i>)	rPen a 1 (TM)	Brown shrimp (<i>P. aztecus</i>)
	rGad c 1 (PV)	Cod (<i>G. morhua</i>)		
Phadia, ImmunoCAP® ISAC	rGad c 1 (PV)	Cod (<i>G. callarias</i>)	nPen m 1 (TM)	Black tiger prawn (<i>P. monodon</i>)
			nPen m 2 (AK)	
			nPen m 4 (SCBP)	
Siemens Immulite®	Not available		rPen m 1 (TM)	Black tiger prawn (<i>P. monodon</i>)

2015). However, further investigation is required into the utility of specific-IgE quantification of one or more component allergens as biomarkers for clinical reactivity to crustaceans.

Currently, mollusc allergens have not been investigated for use in component-resolved diagnostics.

Detailed diagnostics are needed to specifically determine the often very narrow range of species a patient may react to, so that specific products can be avoided, improving the safety of the patient and eliminating the need to abstain from seafood entirely.

10. Therapeutic development for seafood allergy - vaccine candidates, hypoallergenic derivatives and clinical trials

Current treatment strategies for seafood allergy are mostly palliative in nature, along with complete avoidance of the offending source, underlining the need for labelling food and detecting seafood allergens. The major challenge in the development of seafood immunotherapy, using either whole extracts or natural purified allergens, is the risk of severe allergic reactions upon administration of the dose via oral, sublingual, intradermal or subcutaneous route in seafood allergic patients. Therapeutic strategies for seafood allergy, and in general for food allergy, are still in the developmental phase. Approaches for fish and shellfish allergy are fundamentally different and are discussed separately. To our best knowledge, all approaches for shellfish allergy are limited to crustaceans.

Alternative treatments of seafood allergy include the use of probiotics and Chinese herbal medicines as shown for shrimp (Schiavi et al., 2011) and fish (Srivastava et al., 2012) allergy in murine models. Interestingly, fish oil has been reported to be useful for allergy prevention (Calder, 2013).

10.1. Vaccine candidates, hypoallergenic derivatives and clinical trials for fish allergy

To date, the most developed immunotherapy for fish allergy is the use of hypoallergenic proteins for antibody binding to mimic their

natural counterparts. The expression of recombinant Cyp c 1 that contained IgE epitopes from other fish species was first described in 2002 (Swoboda et al., 2002a). This was followed by a study in 2007, which expressed recombinant Cyp c 1 with mutations in either one or both of the CD- or EF-calcium-binding sites, demonstrating that these mutations could reduce the binding to patients IgE by as much as 95% (Swoboda et al., 2007). These mutations inhibited the association of Ca²⁺ due to the conformational alteration in the binding sites (Bugajska-Schretter et al., 2000), which resulted in the loss of the IgE epitope. The allergenicity of the protein was also diminished, confirmed by *in vivo* SPT and *in vitro* basophil histamine release, while maintaining its immunogenic capability to raise IgG to block the subsequently introduced parvalbumin from binding to IgE (Swoboda et al., 2007). The first comprehensive study using a murine model of fish allergy also demonstrated a reduction of the Th2-response by recombinant Cyp c 1 (van der Ventel et al., 2011). Since 2008, this approach using the mutated hypoallergenic parvalbumin has gained more attention and been further explored for its potential. The European Union-funded Food-Allergy Specific ImmunoTherapy (FAST) project is a multi-national, multi-institutional project that aims to combat food allergy by developing safe and efficacious allergen-specific subcutaneous immunotherapies using recombinant hypoallergenic proteins instead of food extracts. As a pre-clinical stage study, the efficacy and reduced allergenicity of recombinant mutated Cyp c 1 were tested *in vivo* using mice and rabbits, and *in vitro* using fish-allergic patients' peripheral blood mononuclear cells, and finally its safety confirmed according to OECD guidelines (Zuidmeer-Jongejan et al., 2015). The first-in-man subcutaneous immunotherapy showed a promising outcome and hence Phase I/IIa clinical trials were conducted with 15 participants in Denmark, 2013 (NCT number: NCT02017626), followed by Phase IIb clinical trial with 45 participants across six countries in 2015 (NCT number: NCT02382718).

More recently, the blocking antibodies produced by immunisation of a murine fish allergy model with the recombinant Cyp c 1 mutant was found to protect from allergic symptoms when used as a passive immunisation (Freidl et al., 2017). The only other candidature for

immunotherapy is based on parvalbumin-specific mimotopes, mimicking the epitope of the respective allergen (Untersmayr et al., 2006). However, to our best knowledge, this approach has not been investigated extensively.

10.2. Vaccine candidates and hypoallergenic derivatives for crustacean allergy

Several studies have focused on the elucidation of B-cell and T-cell epitopes of crustacean allergens. Knowledge on these epitopes is central to the development of peptide- or protein-based vaccines which have abolished or reduced IgE reactivity, and hence no IgE-mediated side effects, but an ability to induce a strong T-cell response. B- and T-cell epitope studies and immunotherapy in animal models are mainly focused on shrimp tropomyosin due to its high clinical relevance. T-cell epitopes on shrimp tropomyosin have been elucidated and experimentally validated using computer algorithms (Subba Rao et al., 1998) or overlapping peptide libraries (Wang et al., 2012). T-cell reactivity has been extensively characterised for heated and raw crustacean species (Abramovitch et al., 2017). Hypoallergenic tropomyosin has been generated with reduced IgE binding through site-directed mutagenesis or truncation/removal of IgE binding epitopes (Reese et al., 2005; Wai et al., 2014), while leaving the T-cell epitopes intact, and tested for desensitising potential in a tropomyosin-sensitised mouse model. T-cell epitope-based peptides have also been investigated as novel immunotherapeutic candidates, demonstrating efficiency in reducing allergic responses towards shrimp tropomyosin in animal studies (Wai et al., 2016). In addition, it has been demonstrated that a low-dose administration of wild-type purified recombinant shrimp tropomyosin can favour a regulatory T-cell response in animal studies as compared to a high-dose regimen (Leung et al., 2017). The efficacy and therapeutic potential of recombinant wild-type and hypoallergenic crustacean allergens have yet to be investigated in clinical trials.

11. Future directions

The identification of allergy-inducing seafood species is often difficult due to the very limited availability of diagnostic methods, complicated by the large diversity of species and limited knowledge of allergens and cross-reactivities. To improve the diagnostics, and subsequent patient care and management, further research needs to be conducted. All clinically relevant allergens from a wide global diversity of species need to be identified and their cross-reactivities need to be investigated. With this gained knowledge, better component-resolved diagnosis based *in vitro* methods can be developed, allowing reliable and precise diagnostics, leading to urgently needed individual management and treatment, including component-resolved immunotherapy.

Recent advances in the field of genomics, bioinformatics, and proteomics have unlocked new opportunities for targeted high-throughput screening and identification of proteins based on amino acid sequence identities, resulting in direct comparative analysis of similar allergens from comprehensive allergen databases. Thus, the combinatorial approach using a specific organism's genome data and comparison with a growing number of comprehensive allergen databases, immune-reactivity, and high throughput protein sequence analysis can overcome the current limitations of allergen identification. This advanced, comprehensive strategy will allow for the development of more sensitive and specific molecular tools for the diagnosis and quantification of seafood allergens. Furthermore, epitope-specific binding, rather than allergen-specific IgE measurements, needs to be considered as it seems to better correlate with clinical reactivity. The current efforts in developing immunotherapeutic approaches for fish and shellfish allergy need to seriously consider the far greater molecular diversity of allergens compared to other food groups.

Acknowledgements

TR is a scholar of the Centre for Food and Allergy Research. RN is a James Cook University Postgraduate Research Scholarship recipient. TL is an Endeavour scholar. SK is an National Health and Medical Research Council (NHMRC) Peter Doherty ECR fellow. AL is supported by funding from the Australian Research Council and the NHMRC.

References

- Abdel Rahman, A.M., Kamath, S.D., Lopata, A.L., Robinson, J.J., Helleur, R.J., 2011. Biomolecular characterization of allergenic proteins in snow crab (*Chionoecetes opilio*) and de novo sequencing of the second allergen arginine kinase using tandem mass spectrometry. *J. Proteom.* 74 (2), 231–241.
- Abramovitch, J.B., Kamath, S., Varese, N., Zubrinich, C., Lopata, A.L., O'Hehir, R.E., Rolland, J.M., 2013. IgE reactivity of blue swimmer crab (*Portunus pelagicus*) tropomyosin, Por p 1, and Other Allergens; cross-reactivity with black tiger prawn and effects of heating. *PLoS One* 8 (6), e67487.
- Abramovitch, J.B., Lopata, A.L., O'Hehir, R.E., Rolland, J.M., 2017. Effect of thermal processing on T cell reactivity of shellfish allergens - discordance with IgE reactivity. *Plos One* 12 (3), e0173549.
- Albrecht, M., Kuhne, Y., Ballmer-Weber, B.K., Becker, W.M., Holzhauser, T., Lauer, I., Reuter, A., Randow, S., Falk, S., Wangorsch, A., Lidholm, J., Reese, G., Vieths, S., 2009. Relevance of IgE binding to short peptides for the allergenic activity of food allergens. *J. Allergy Clin. Immunol.* 124 (2), 328–336 e321–326.
- Amaral, L., Raposo, A., Morais, Z., Coimbra, A., 2018. Jellyfish ingestion was safe for patients with crustaceans, cephalopods, and fish allergy. *Asia Pac. Allergy* 8 (1).
- Anand, S., Kamath, S., Chuang, L.L., Kasapis, S., Lopata, A.L., 2013. Biochemical and thermo-mechanical analysis of collagen from the skin of Asian Sea bass (*Lates calcarifer*) and Australasian Snapper (*Pagrus auratus*), an alternative for mammalian collagen. *Eur. Food Res. Technol.* 236 (5), 873–882.
- Arif, S.H., 2009. A Ca(2+)-binding protein with numerous roles and uses: parvalbumin in molecular biology and physiology. *BioEssays* 31 (4), 410–421.
- Arlan, L.G., Morgan, M.S., Vyszynski-Moher, D.L., Sharra, D., 2009. Cross-reactivity between storage and dust mites and between mites and shrimp. *Exp. Appl. Acarol.* 47 (2), 159–172.
- Arnold, K., Bordoli, L., Kopp, J., Schwede, T., 2006. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Biog. Amines* 22 (2), 195–201.
- Asero, R., Mistrello, G., Roncarolo, D., Casarini, M., Falagiani, P., 1999. True mono-sensitivity to a tropical sole. *Allergy* 54 (11), 1228–1229.
- Asturias, J.A., Eraso, E., Arilla, M.C., Gomez-Bayon, N., Inacio, F., Martinez, A., 2002. Cloning, isolation, and IgE-binding properties of *Helix aspersa* (brown garden snail) tropomyosin. *Int. Arch. Allergy Immunol.* 128 (2), 90–96.
- Ayuso, R., Grishina, G., Bardina, L., Carrillo, T., Blanco, C., Ibanez, M.D., Sampson, H.A., Beyer, K., 2008. Myosin light chain is a novel shrimp allergen, Lit v 3. *J. Allergy Clin. Immunol.* 122 (4), 795–802.
- Ayuso, R., Grishina, G., Ibanez, M.D., Blanco, C., Carrillo, T., Bencharitwong, R., Sanchez, S., Nowak-Wegrzyn, A., Sampson, H.A., 2009. Sarcoplasmic calcium-binding protein is an EF-hand-type protein identified as a new shrimp allergen. *J. Allergy Clin. Immunol.* 124 (1), 114–120.
- Ayuso, R., Reese, G., Leong-Kee, S., Plante, M., Lehrer, S.B., 2002. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. *Int. Arch. Allergy Immunol.* 129 (1), 38–48.
- Ayuso, R., Sánchez-García, S., Lin, J., Fu, Z., Ibáñez, M.D., Carrillo, T., Blanco, C., Goldis, M., Bardina, L., Sastre, J., 2010. Greater epitope recognition of shrimp allergens by children than by adults suggests that shrimp sensitization decreases with age. *J. Allergy Clin. Immunol.* 125 (6), 1286–1293 e1283.
- Azzi, A., Clark, S.A., Ellington, W.R., Chapman, M.S., 2004. The role of phosphagen specificity loops in arginine kinase. *Protein Sci.* 13 (3), 575–585.
- Baird, F.J., Gasser, R.B., Jabbar, A., Lopata, A.L., 2014. Foodborne anisakiasis and allergy. *Mol. Cell Probes* 28 (4), 167–174.
- Bauermeister, K., Wangorsch, A., Garoffo, L.P., Reuter, A., Conti, A., Taylor, S.L., Lidholm, J., Dewitt, A.M., Enrique, E., Vieths, S., Holzhauser, T., Ballmer-Weber, B., Reese, G., 2011. Generation of a comprehensive panel of crustacean allergens from the North Sea Shrimp Crangon Crangon. *Mol. Immunol.* 48 (15–16), 1983–1992.
- Beale, J.E., Jeebhay, M.F., Lopata, A.L., 2009. Characterisation of purified parvalbumin from five fish species and nucleotide sequencing of this major allergen from Pacific pilchard, *Sardinops sagax*. *Mol. Immunol.* 46 (15), 2985–2993.
- Behrmann, E., Muller, M., Penczek, P.A., Mannherz, H.G., Manstein, D.J., Raunser, S., 2012. Structure of the rigor actin-tropomyosin-myosin complex. *Cell* 150 (2), 327–338.
- Ben-Shoshan, M., Harrington, D.W., Solter, L., Fragapane, J., Joseph, L., St Pierre, Y., Godefroy, S.B., Elliott, S.J., Clarke, A.E., 2010. A population-based study on peanut, tree nut, fish, shellfish, and sesame allergy prevalence in Canada. *J. Allergy Clin. Immunol.* 125 (6), 1327–1335.
- Bessot, J.C., Metz-Favre, C., Rame, J.M., De Blay, F., Pauli, G., 2010. Tropomyosin or not tropomyosin, what is the relevant allergen in house dust mite and snail cross allergies? *Eur. Ann. Allergy Clin. Immunol.* 42 (1), 3–10.
- Biji, K.B., Ravishankar, C.N., Venkateswarlu, R., Mohan, C.O., Gopal, T.K., 2016. Biogenic amines in seafood: a review. *J. Food Sci. Technol.* 53 (5), 2210–2218.
- Binder, M., Mahler, V., Hayek, B., Sperr, W.R., Scholler, M., Prozell, S., Wiedermann, G., Valent, P., Valenta, R., Duchene, M., 2001. Molecular and immunological

- characterization of arginine kinase from the Indianmeal moth, *Plodia interpunctella*, a novel cross-reactive invertebrate pan-allergen. *J. Immunol.* 167 (9), 5470–5477.
- Bobolea, I., Barranco, P., Pastor-Vargas, C., Iraola, V., Vivanco, F., Quirce, S., 2011. Arginine kinase from the cellar spider (*Holocnemus pluchei*): a new asthma-causing allergen. *Int. Arch. Allergy Immunol.* 155 (2), 180–186.
- Boquete, M., Iraola, V., Morales, M., Pinto, H., Francisco, C., Carballas, C., Carnes, J., 2011. Seafood hypersensitivity in mite sensitized individuals: is tropomyosin the only responsible allergen? *Ann. Allergy Asthma Immunol.* 106 (3), 223–229.
- Brownridge, P., de Mello, L.V., Peters, M., McLean, L., Claydon, A., Cossins, A.R., Whitfield, P.D., Young, I.S., 2009. Regional variation in parvalbumin isoform expression correlates with muscle performance in common carp (*Cyprinus carpio*). *J. Exp. Biol.* 212 (Pt. 2), 184–193.
- Bugajska-Schretter, A., Elfman, L., Fuchs, T., Kapiotis, S., Rumpold, H., Valenta, R., Spitzauer, S., 1998. Parvalbumin, a cross-reactive fish allergen, contains IgE-binding epitopes sensitive to periodate treatment and Ca^{2+} depletion. *J. Allergy Clin. Immunol.* 101 (Pt. 1), 67–74.
- 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 allergen: different immunoglobulin E recognition of the apo- and calcium-bound forms of carp parvalbumin. *Gut* 46 (5), 661–669.
- Burney, P., Summers, C., Chinn, S., Hooper, R., van Ree, R., Lidholm, J., 2010. Prevalence and distribution of sensitization to foods in the European Community Respiratory Health Survey: a EuroPrevall analysis. *Allergy* 65 (9), 1182–1188.
- Cai, Q.-F., Liu, G.-M., Li, T., Hara, K., Wang, X.-C., Su, W.-J., Cao, M.-J., 2010. Purification and characterization of parvalbumins, the major allergens in red stingray (*Dasyatis akajei*). *J. Agric. Food. Chem.* 58 (24), 12964–12969.
- Cain, J.A., Solis, N., Cordwell, S.J., 2014. Beyond gene expression: the impact of protein post-translational modifications in bacteria. *J. Proteom.* 97, 265–286.
- Calder, P.C., 2013. Fishing for allergy prevention. *Clin. Exp. Allergy* 43 (7), 700–702.
- Campbell, B., Pauly, D., 2013. Mariculture: a global analysis of production trends since 1950. *Mar. Policy* 39, 94–100.
- Carrillo, T., Castillo, R., Caminero, J., Cuevas, M., Rodriguez, J.C., Acosta, O., Rodriguez de Castro, F., 1992. Squid hypersensitivity: a clinical and immunologic study. *Ann. Allergy* 68 (6), 483–487.
- Cates, M.S., Berry, M.B., Ho, E.L., Li, Q., Potter, J.D., Phillips Jr., G.N., 1999. Metal-ion affinity and specificity in EF-hand proteins: coordination geometry and domain plasticity in parvalbumin. *Structure* 7 (10), 1269–1278.
- Chao, E., Kim, H.W., Mykles, D.L., 2010. Cloning and tissue expression of eleven tropomyosin-C isoforms in the American lobster, *Homarus americanus*. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 157 (1), 88–101.
- Chen, H.L., Cao, M.J., Cai, Q.F., Su, W.J., Mao, H.Y., Liu, G.M., 2013a. Purification and characterisation of sarcoplasmic calcium-binding protein, a novel allergen of red swamp crayfish (*Procambarus clarkii*). *Food Chem.* 139 (1–4), 213–223.
- Chen, H.L., Mao, H.Y., Cao, M.J., Cai, Q.F., Su, W.J., Zhang, Y.X., Liu, G.M., 2013b. Purification, physicochemical and immunological characterization of arginine kinase, an allergen of crayfish (*Procambarus clarkii*). *Food Chem. Toxicol.* 62, 475–484.
- Chen, L., Hefle, S.L., Taylor, S.L., Swoboda, I., Goodman, R.E., 2006. Detecting fish parvalbumin with commercial mouse monoclonal anti-frog parvalbumin IgG. *J. Agr. Food Chem.* 54 (15), 5577–5582.
- Christova, P., Cox, J.A., Craescu, C.T., 2000. Ion-induced conformational and stability changes in Nereis sarcoplasmic calcium binding protein: evidence that the APO state is a molten globule. *Proteins: Struct., Funct., Genet.* 40 (2), 177–184.
- Chu, K.H., Wong, S.H., Leung, P.S., 2000. Tropomyosin is the major mollusk allergen: reverse transcriptase polymerase chain reaction, expression and IgE reactivity. *Mar. Biotechnol.* (NY) 2 (5), 499–509.
- Chuang, J.G., Su, S.N., Chiang, B.L., Lee, H.J., Chow, L.P., 2010. Proteome mining for novel IgE-binding proteins from the German cockroach (*Blattella germanica*) and allergen profiling of patients. *Proteomics* 10 (21), 3854–3867.
- Clark, S.A., Davulcu, O., Chapman, M.S., 2012. Crystal structures of arginine kinase in complex with ADP, nitrate, and various phosphagen analogs. *Biochem. Biophys. Res. Commun.* 427 (1), 212–217.
- Connett, G.J., Gerez, I., Cabrera-Morales, E.A., Yuenyongviwat, A., Ngamphaiboon, J., Chatchatee, P., Sangsupawanich, P., Soh, S.E., Yap, G.C., Shek, L.P., Lee, B.W., 2012. A population-based study of fish allergy in the Philippines, Singapore and Thailand. *Int. Arch. Allergy Immunol.* 159 (4), 384–390.
- Cook, W.J., Jeffrey, L.C., Cox, J.A., Vijay-Kumar, S., 1993. Structure of a sarcoplasmic calcium-binding protein from amphioxus refined at 2.4 Å resolution. *J. Mol. Biol.* 229 (2), 461–471.
- Das Dore, S., Chopin, C., Romano, A., Galland-Irmouli, A.V., Quarantino, D., Pascual, C., Fleurence, J., Gueant, J.L., 2002. IgE-binding and cross-reactivity of a new 41 kDa allergen of codfish. *Allergy* 57 (Suppl 72), 84–87.
- Daul, C.B., Slattery, M., Reese, G., Lehrer, S.B., 1994. Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *Int. Arch. Allergy Immunol.* 105 (1), 49–55.
- de Jongh, H.H., Taylor, S.L., Koppelman, S.J., 2011. Controlling the aggregation propensity and thereby digestibility of allergens by Maillardation as illustrated for cod fish parvalbumin. *J. Biosci. Bioeng.* 111 (2), 204–211.
- De Nicola, G., Burkart, C., Qiu, F., Agianian, B., Labeit, S., Martin, S., Bullard, B., Pastore, A., 2007. The structure of Lethocerus tropomyosin C: insights into the mechanism of stretch activation in muscles. *Structure* 15 (7), 813–824.
- Declercq, J.P., Evrard, C., Lamzin, V., Parello, J., 1999. Crystal structure of the EF-hand parvalbumin at atomic resolution (0.91 Å) and at low temperature (100 K). Evidence for conformational multistates within the hydrophobic core. *Protein Sci.* 8 (10), 2194–2204.
- Declercq, J.P., Ninant, B., Parello, J., 1996. X-ray structure of a new crystal form of pike 4.10 beta parvalbumin. *Acta Crystallogr. D Biol. Crystallogr.* 52 (Pt. 1), 165–169.
- Declercq, J.P., Ninant, B., Parello, J., Rambaud, J., 1991. Ionic interactions with parvalbumins. Crystal structure determination of pike 4.10 parvalbumin in four different ionic environments. *J. Mol. Biol.* 220 (4), 1017–1039.
- Dietrich, M.A., Westfalewicz, B., Jurecka, P., Imrazarow, I., Ciereszko, A., 2014. Isolation, characterisation and cDNA sequencing of a new form of parvalbumin from carp semen. *Reprod. Fertil. Dev.* 26 (8), 1117–1128.
- Ebo, D.G., Kuehn, A., Britts, C.H., Hilger, C., Hentges, F., Stevens, W.J., 2010. Monosensitivity to pangasius and tilapia caused by allergens other than parvalbumin. *J. Invest. Allerg. Clin.* 20 (1), 84–88.
- Elsayed, S., Apold, J., 1983. Immunochemical analysis of cod fish allergen M: locations of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. *Allergy* 38 (7), 449–459.
- Emoto, A., Ishizaki, S., Shiomi, K., 2009. Tropomyosins in gastropods and bivalves: identification as major allergens and amino acid sequence features. *Food Chem.* 114 (2), 634–641.
- Fohr, U.G., Weber, B.R., Muntener, M., Staudenmann, W., Hughes, G.J., Frutiger, S., Banville, D., Schafer, B.W., Heizmann, C.W., 1993. Human alpha and beta parvalbumins. Structure and tissue-specific expression. *Eur. J. Biochem.* 215 (3), 719–727.
- Freemont, P.S., Dunbar, B., Fothergill-Gilmore, L.A., 1988. The complete amino acid sequence of human skeletal-muscle fructose-bisphosphate aldolase. *Biochem. J.* 249 (3), 779–788.
- Freidl, R., Gstoettner, A., Baranyi, U., Swoboda, I., Stolz, F., Focke-Tejkl, M., Wekerle, T., van Ree, R., Valenta, R., Linhart, B., 2017. Blocking antibodies induced by immunization with a hypoallergenic parvalbumin mutant reduce allergic symptoms in a mouse model of fish allergy. *J. Allergy Clin. Immunol.* 139 (6), 1897–1905 e1891.
- Friedman, M.A., Fernandez, M., Backer, L.C., Dickey, R.W., Bernstein, J., Schrank, K., Kibler, S., Stephan, W., Gribble, M.O., Bienfang, P., Bowen, R.E., Degrasse, S., Flores Quintana, H.A., Loeffler, C.R., Weisman, R., Blythe, D., Berdalet, E., Ayyar, R., Clarkson-Townsend, D., Swajian, K., Benner, R., Brewer, T., Fleming, L.E., 2017. An updated review of ciguatera fish poisoning: clinical, epidemiological, environmental, and public health management. *Mar. Drugs* 15 (3), 72.
- Gajewski, K.G., Hsieh, Y.H., 2009. Monoclonal antibody specific to a major fish allergen: parvalbumin. *J. Food Prot.* 72 (4), 818–825.
- Gamez, C., Sanchez-Garcia, S., Ibanez, M.D., Lopez, R., Aguado, E., Lopez, E., Sastre, B., Sastre, J., del Pozo, V., 2011. Tropomyosin IgE-positive results are a good predictor of shrimp allergy. *Allergy* 66 (10), 1375–1383.
- Gamez, C., Zafra, M.P., Sanz, V., Mazzeo, C., Ibanez, M.D., Sastre, J., del Pozo, V., 2015. Simulated gastrointestinal digestion reduces the allergic reactivity of shrimp extract proteins and tropomyosin. *Food Chem.* 173, 475–481.
- Garcia-Orozco, K.D., Aispuro-Hernandez, E., Yepiz-Plascencia, G., Calderon-de-la-Barca, A.M., Sotelo-Mundo, R.R., 2007. Molecular characterization of arginine kinase, an allergen from the shrimp *Litopenaeus vannamei*. *Int. Arch. Allergy Immunol.* 144 (1), 23–28.
- Garone, L., Theibert, J.L., Miegel, A., Maeda, Y., Murphy, C., Collins, J.H., 1991. Lobster tropomyosin C: amino acid sequences of three isoforms. *Arch. Biochem. Biophys.* 291 (1), 89–91.
- Gattis, J.L., Ruben, E., Fenley, M.O., Ellington, W.R., Chapman, M.S., 2004. The active site cysteine of arginine kinase: structural and functional analysis of partially active mutants. *Biochemistry* 43 (27), 8680–8689.
- Gerday, C., 1976. The primary structure of the parvalbumin II of pike (*Esox lucius*). *Eur. J. Biochem.* 70 (1), 305–318.
- Gillis, J.M., 1985. Relaxation of vertebrate skeletal muscle. A synthesis of the biochemical and physiological approaches. *Biochim. Biophys. Acta* 811 (2), 97–145.
- Gistelinc, C., Gioia, R., Gagliardi, A., Tonelli, F., Marchese, L., Bianchi, L., Landi, C., Bini, L., Huysseune, A., Witten, P.E., Staes, A., Gevaert, K., De Rocker, N., Menten, B., Malfait, F., Leikin, S., Carra, S., Tenni, R., Rossi, A., De Paepe, A., Coucke, P., Willaert, A., Forlino, A., 2016. Zebrafish collagen Type I: molecular and biochemical characterization of the major structural protein in bone and skin. *Sci. Rep.* 6, 21540.
- Giuffrida, M.G., Villalta, D., Mistrello, G., Amato, S., Asero, R., 2014. Shrimp allergy beyond Tropomyosin in Italy: clinical relevance of Arginine Kinase, Sarcoplasmic calcium binding protein and Hemocyanin. *Eur. Ann. Allergy Clin. Immunol.* 46 (5), 172–177.
- Godsey, M.H., Davulcu, O., Nix, J.C., Skalicky, J.J., Bruschweiler, R.P., Chapman, M.S., 2016. The sampling of conformational dynamics in ambient-temperature crystal structures of arginine kinase. *Structure* 24 (10), 1658–1667.
- Goetz, D.W., Whisman, B.A., 2000. Occupational asthma in a seafood restaurant worker: cross-reactivity of shrimp and scallops. *Ann. Allergy Asthma Immunol.* 85 (Pt. 6), 461–466.
- Gonzalez-Fernandez, J., Alguacil-Guillen, M., Cuellar, C., Daschner, A., 2018. Possible allergenic role of tropomyosin in patients with adverse reactions after fish intake. *Immunol. Invest.* 1–14.
- Gonzalez-Fernandez, J., Daschner, A., Cuellar, C., 2017. Allergenicity of vertebrate tropomyosins: challenging an immunological dogma. *Allergol. Immunopathol. (Madr)* 45 (3), 297–304.
- Gonzalez-Fernandez, J., Veleiro, B., Daschner, A., Cuellar, C., 2016. Are fish tropomyosins allergens? *Ann. Allergy Asthma Immunol.* 116 (1), 74–76 e75.
- Griesmeier, U., Bublin, M., Radauer, C., Vazquez-Cortes, S., Ma, Y., Fernandez-Rivas, M., Breiteneder, H., 2010a. Physicochemical properties and thermal stability of Lep w 1, the major allergen of whiff. *Mol. Nutr. Food Res* 54 (6), 861–869.
- Griesmeier, U., Vazquez-Cortes, S., Bublin, M., Radauer, C., Ma, Y., Briza, P., Fernandez-Rivas, M., Breiteneder, H., 2010b. Expression levels of parvalbumins determine allergenicity of fish species. *Allergy* 65 (2), 191–198.
- Guo, F.F., Kubota, H., Shiomi, K., 2012. Purification, immunological properties and molecular cloning of two allergenic parvalbumins from the crimson sea bream, *Eyynniss japonica*. *Food Chem.* 132 (2), 835–840.

- Hamada, Y., Nagashima, Y., Shiomi, K., 2001. Identification of collagen as a new fish allergen. *Biosci. Biotech. Biochem.* 65 (2), 285–291.
- Hamada, Y., Nagashima, Y., Shiomi, K., Shimojo, N., Kohno, Y., Shibata, R., Nishima, S., Ohsuna, H., Ikezawa, Z., 2003. Reactivity of IgE in fish-allergic patients to fish muscle collagen. *Allergol. Int.* 52 (3), 139–147.
- Hamada, Y., Tanaka, H., Sato, A., Ishizaki, S., Nagashima, Y., Shiomi, K., 2004. Expression and evaluation of IgE-binding capacity of recombinant Pacific mackerel parvalbumin. *Allergol. Int.* 53 (3), 271–278.
- Heizmann, C.W., 1984. Parvalbumin, an intracellular calcium-binding protein; distribution, properties and possible roles in mammalian cells. *Experientia* 40 (9), 910–921.
- Henzl, M.T., Tanner, J.J., 2007. Solution structure of Ca²⁺-free rat beta-parvalbumin (oncomodulin). *Protein Sci.* 16 (9), 1914–1926.
- Henzl, M.T., Tanner, J.J., Tan, A., 2011. Solution structures of chicken parvalbumin 3 in the Ca(2+)-free and Ca(2+)-bound states. *Proteins* 79 (3), 752–764.
- Hoppe, S., Steinhart, H., Paschke, A., 2006. Identification of a 28 kDa lychee allergen as a triose-phosphate isomerase. *Food Agric. Immunol.* 17 (1), 9–19.
- Hu, M.J., Liu, G.Y., Yang, Y., Pan, T.M., Liu, Y.X., Sun, L.C., Cao, M.J., Liu, G.M., 2017. Cloning, expression, and the effects of processing on sarcoplasmic-calcium-binding protein: an important allergen in mud crab. *J. Agric. Food Chem.* 65 (30), 6247–6257.
- Huang, M.C., Ochiai, Y., 2005. Fish fast skeletal muscle tropomyosins show species-specific thermal stability. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 141 (4), 461–471.
- Imamura, K., Tsuruta, D., Tsuchisaka, A., Mori, T., Ohata, C., Furumura, M., Hashimoto, T., 2013. Anaphylaxis caused by ingestion of jellyfish. *Eur. J. Dermatol.* 23 (3), 392–395.
- Ishikawa, M., Ishida, M., Shimakura, K., Nagashima, Y., Shiomi, K., 1998a. Purification and IgE-binding epitopes of a major allergen in the gastropod *Turbo cornutus*. *Biosci. Biotech. Biochem.* 62 (7), 1337–1343.
- Ishikawa, M., Ishida, M., Shimakura, K., Nagashima, Y., Shiomi, K., 1998b. Tropomyosin, the major oyster *Crassostrea gigas* allergen and its IgE-binding epitopes. *J. Food Sci.* 63 (1), 44–47.
- Ishikawa, M., Shimakura, K., Nagashima, Y., Shiomi, K., 1997. Isolation and properties of allergenic proteins in the oyster *Crassostrea gigas*. *Fish. Sci.* 63 (4), 610–614.
- Ishikawa, M., Suzuki, F., Ishida, M., Nagashima, Y., Shiomi, K., 2001. Identification of tropomyosin as a major allergen in the octopus *Octopus vulgaris* and elucidation of its IgE-binding epitopes. *Fish. Sci.* 67 (5), 934–942.
- Jackman, D.M., Waddleton, D.M., Youngband, B., Heeley, D.H., 1996. Further characterisation of fast, slow and cardiac muscle tropomyosins from salmonid fish. *Eur. J. Biochem.* 242 (2), 363–371.
- Jarilla, B.R., Uda, K., Suzuki, T., Acosta, L.P., Urabe, M., Agatsuma, T., 2014. Characterization of arginine kinase from the caenogastropod *semisulcospira libertina*, an intermediate host of *paragonimus westermani*. *J. Mollus. Stud.* 80 (4), 444–451.
- Jeebhay, M.F., Lopata, A.L., 2012. Occupational allergies in seafood-processing workers. *Advances in Food and Nutrition Research*, vol. 66. Elsevier, pp. 47–73.
- Jenkins, J.A., Breiteneder, H., Mills, E.N., 2007. Evolutionary distance from human homologs reflects allergenicity of animal food proteins. *J. Allergy Clin. Immunol.* 120 (6), 1399–1405.
- Jimenez, M., Pineda, F., Sanchez, I., Orozco, I., Senent, C., 2005. Allergy due to *Ensis macha*. *Allergy* 60 (8), 1090–1091.
- Jin, Y., Deng, Y., Qian, B., Zhang, Y., Liu, Z., Zhao, Y., 2015. Allergic response to squid (*Todarodes pacificus*) tropomyosin *Tod p1* structure modifications induced by high hydrostatic pressure. *Food Chem. Toxicol.* 76, 86–93.
- Johnston, E.B., Kamath, S.D., Lopata, A.L., Schaeffer, P.M., 2014. Tus-Ter-lock immuno-PCR assays for the sensitive detection of tropomyosin-specific IgE antibodies. *Bioanalysis* 6 (4), 465–476.
- Kalyanasundaram, A., Santiago, T.C., 2015. Identification and characterization of new allergen tropomyosin C (Pen m 6.0101) from Indian black tiger shrimp *Penaeus monodon*. *Eur. Food Res. Technol.* 240 (3), 509–515.
- Kamath, S.D., Abdel Rahman, A.M., Komoda, T., Lopata, A.L., 2013. Impact of heat processing on the detection of the major shellfish allergen tropomyosin in crustaceans and molluscs using specific monoclonal antibodies. *Food Chem.* 141 (4), 4031–4039.
- Kamath, S.D., Johnston, E.B., Iyer, S., Schaeffer, P.M., Koplin, J., Allen, K., Lopata, A.L., 2017. IgE reactivity to shrimp allergens in infants and their cross-reactivity to house dust mite. *Pediatr. Allergy Immunol.* 28 (7), 703–707.
- Kamath, S.D., Rahman, A.M., Voskamp, A., Komoda, T., Rolland, J.M., O'Hehir, R.E., Lopata, A.L., 2014a. Effect of heat processing on antibody reactivity to allergen variants and fragments of black tiger prawn: a comprehensive allergenomic approach. *Mol. Nutr. Food Res.* 58 (5), 1144–1155.
- Kamath, S.D., Thomassen, M.R., Saptarshi, S.R., Nguyen, H.M., Aasmoe, L., Bang, B.E., Lopata, A.L., 2014b. Molecular and immunological approaches in quantifying the airborne food allergen tropomyosin in crab processing facilities. *Int. J. Hyg. Environ. Health* 217 (7), 740–750.
- Kamdar, T.A., Peterson, S., Lau, C.H., Saltoun, C.A., Gupta, R.S., Bryce, P.J., 2015. Prevalence and characteristics of adult-onset food allergy. *J. Allergy Clin. Immunol. Pract.* 3 (1), 114–115 e111.
- Kantha, S.S., Watabe, S., Hashimoto, K., 1990. Comparative biochemistry of paramyosin? A review. *J. Food Biochem.* 14 (1), 61–88.
- Kelso, J.M., Jones, R.T., Yunginger, J.W., 1996. Monospecific allergy to swordfish. *Ann. Allergy Asthma Immunol.* 77 (3), 227–228.
- Khanarukombat, S., Srisomsap, C., Chokchaichamnankit, D., Punyavit, P., Phiriyangkul, P., 2014. Identification of a novel allergen from muscle and various organs in banana shrimp (*Fenneropenaeus merguensis*). *Ann. Allergy Asthma Immunol.* 113 (3), 301–306.
- Khurana, T., Collison, M., Chew, F.T., Slater, J.E., 2014. Bla g 3: a novel allergen of German cockroach identified using cockroach-specific avian single-chain variable fragment antibody. *Ann. Allergy Asthma Immunol.* 112 (2), 140–145 e141.
- Kobayashi, A., Kobayashi, Y., Shiomi, K., 2016a. Fish allergy in patients with parvalbumin-specific immunoglobulin E depends on parvalbumin content rather than molecular differences in the protein among fish species. *Biosci. Biotech. Biochem.* 80 (10), 2018–2021.
- Kobayashi, A., Tanaka, H., Hamada, Y., Ishizaki, S., Nagashima, Y., Shiomi, K., 2006. Comparison of allergenicity and allergens between fish white and dark muscles. *Allergy* 61 (3), 357–363.
- Kobayashi, Y., Akiyama, H., Hige, J., Kubota, H., Chikazawa, S., Satoh, T., Miyake, T., Uhara, H., Okuyama, R., Nakagawara, R., Aihara, M., Hamada-Sato, N., 2016b. Fish collagen is an important panallergen in the Japanese population. *Allergy* 71 (5), 720–723.
- Kobayashi, Y., Hige, J., Imamura, S., Hamada-Sato, N., 2016c. Study of the cross-reactivity of fish allergens based on a questionnaire and blood testing. *Allergol. Int.* 65 (3), 272–279.
- Kobayashi, Y., Yang, T., Yu, C.T., Ume, C., Kubota, H., Shimakura, K., Shiomi, K., Hamada-Sato, N., 2016d. Quantification of major allergen parvalbumin in 22 species of fish by SDS-PAGE. *Food Chem.* 194, 345–353.
- Koeberl, M., Kamath, S.D., Saptarshi, S.R., Smout, M.J., Rolland, J.M., O'Hehir, R.E., Lopata, A.L., 2014. Auto-induction for high yield expression of recombinant novel isoallergen tropomyosin from King prawn (*Melicerus laticulatus*) for improved diagnostics and immunotherapeutics. *J. Immunol. Methods* 415, 6–16.
- Kondo, Y., Komatsubara, R., Nakajima, Y., Kawamura, M., Kakami, M., Tsuge, I., Yasuda, T., Urisu, A., 2006. Parvalbumin was not responsible for the cross-reactivity between tuna and marlin: a case report. *J. Allergy Clin. Immun.* 117 (2), S50.
- Koppelman, S.J., Nordlee, J.A., Lee, P.W., Happe, R.P., Hessing, M., Norland, R., Manning, T., Deschene, R., De Jong, G.A., Taylor, S.L., 2012. Parvalbumin in fish skin-derived gelatin: is there a risk for fish allergic consumers? *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 29 (9), 1347–1355.
- Koyama, H., Kakami, M., Kawamura, M., Tokuda, R., Kondo, Y., Tsuge, I., Yamada, K., Yasuda, T., Urisu, A., 2006. Grades of 43 fish species in Japan based on IgE-binding activity. *Allergol. Int.* 55 (3), 311–316.
- Kubota, H., Kobayashi, A., Kobayashi, Y., Shiomi, K., Hamada-Sato, N., 2016. Reduction in IgE reactivity of Pacific mackerel parvalbumin by heat treatment. *Food Chem.* 206, 78–84.
- Kuehn, A., Codreanu-Morel, F., Lehnert-Weber, C., Doyen, V., Gomez-André, S.A., Biennu, F., Fischer, J., Ballardini, N., Hage, M., Perotin, J.M., 2016. Cross-reactivity to fish and chicken meat—a new clinical syndrome. *Allergy*.
- Kuehn, A., Hilger, C., Lehnert-Weber, C., Codreanu-Morel, F., Morisset, M., Metz-Favre, C., Pauli, G., de Blay, F., Revets, D., Muller, C.P., Vogel, L., Vieths, S., Hentges, F., 2013. 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* 43 (7), 811–822.
- Kuehn, A., Hutt-Kempf, E., Hilger, C., Hentges, F., 2011. Clinical monosensitivity to salmonid fish linked to specific IgE-epitopes on salmon and trout beta-parvalbumins. *Allergy* 66 (2), 299–301.
- Kuehn, A., Scheuermann, T., Hilger, C., Hentges, F., 2010. Important variations in parvalbumin content in common fish species: a factor possibly contributing to variable allergenicity. *Int. Arch. Allergy Immunol.* 153 (4), 359–366.
- Kuehn, A., Swoboda, I., Arumugam, K., Hilger, C., Hentges, F., 2014. Fish allergens at a glance: variable allergenicity of parvalbumins, the major fish allergens. *Front. Immunol.* 5, 179.
- Kumar, V.D., Lee, L., Edwards, B.F., 1990. Refined crystal structure of calcium-liganded carp parvalbumin 4.25 at 1.5-Å resolution. *Biochemistry* 29 (6), 1404–1412.
- Kumeta, H., Nakayama, H., Ogura, K., 2017. Solution structure of the major fish allergen parvalbumin *Sco j 1* derived from the Pacific mackerel. *Sci. Rep.* 7 (1), 17160.
- Kumjij, S., Jirapongsananurak, O., Piboonpocanun, S., 2016. Cloning and characterization of recombinant tropomyosin of giant freshwater shrimp *M. rosenbergii* to determine major allergens causing allergic reactions among shrimp-allergic children. *Asian Pac. J. Allergy Immunol.* 34 (3), 229–235.
- Kunimoto, A., Sisino, T., Sakai, K., Matsumoto, T., Takahashi, K., Yamashita, H., Hiemori, M., Tsuji, H., Kimoto, M., 2009. Molecular cloning and allergenicity of Pen j 1, a major allergen of kuruma prawn, *Penaeus japonicus*. *Biosci. Biotech. Biochem.* 73 (4), 840–848.
- Lam, Y.F., Tong, K.K., Kwan, K.M., Tsuneyama, K., Shu, S.A., Leung, P.S., Chu, K.H., 2015. Gastrointestinal immune response to the shrimp allergen tropomyosin: histological and immunological analysis in an animal model of shrimp tropomyosin hypersensitivity. *Int. Arch. Allergy Immunol.* 167 (1), 29–40.
- Lao-araya, M., Trakultivakorn, M., 2012. Prevalence of food allergy among preschool children in northern Thailand. *Pediatr. Int.* 54 (2), 238–243.
- Larco-Rojas, X., González-Gutiérrez, M., Vázquez-Cortés, S., Bartolomé, B., Pastor-Vargas, C., Fernández-Rivas, M., 2017. Occupational asthma and urticaria in a fishmonger due to creatine kinase, a cross-reactive fish allergen. *J. Invest. Allergol. Clin. Immunol.* 27 (6), 386.
- Lasekan, A., Cao, H., Maleki, S., Nayak, B.B., 2017. Shrimp tropomyosin retains antibody reactivity after exposure to acidic condition. *J. Sci. Food Agric.* 97 (11), 3623–3630.
- Lee, A.J., Gerez, I., Shek, L.P., Lee, B.W., 2012a. Shellfish allergy—an Asia-Pacific perspective. *Asian Pac. J. Allergy Immunol.* 30 (1), 3–10.
- Lee, C.S., Tsai, L.C., Chao, P.L., Lin, C.Y., Hung, M.W., Chien, A.I., Chiang, Y.T., Han, S.H., 2004. Protein sequence analysis of a novel 103-kDa *Dermatophagoides pteronyssinus* mite allergen and prevalence of serum immunoglobulin E reactivity to rDer p 11 in allergic adult patients. *Clin. Exp. Allergy* 34 (3), 354–362.
- Lee, P.W., Nordlee, J.A., Koppelman, S.J., Baumert, J.L., Taylor, S.L., 2011. Evaluation and comparison of the species-specificity of 3 anti-parvalbumin IgG antibodies. *J. Agric. Food Chem.* 59 (23), 12309–12316.
- Lee, P.W., Nordlee, J.A., Koppelman, S.J., Baumert, J.L., Taylor, S.L., 2012b. Measuring

- parvalbumin levels in fish muscle tissue: relevance of muscle locations and storage conditions. *Food Chem.* 135 (2), 502–507.
- Lee, S.U., Song, H.O., Lee, W., Singaravelu, G., Yu, J.R., Park, W.Y., 2009. Identification and characterization of a putative basic helix-loop-helix (bHLH) transcription factor interacting with calcineurin in *C. elegans*. *Mol. Cells* 28 (5), 455–461.
- Lee, S.Y., Lee, B.L., Soderhall, K., 2003. Processing of an antibacterial peptide from hemocyanin of the freshwater crayfish *Pacifastacus leniusculus*. *J. Biol. Chem.* 278 (10), 7927–7933.
- Lees-Miller, J.P., Helfman, D.M., 1991. The molecular basis for tropomyosin isoform diversity. *BioEssays* 13 (9), 429–437.
- Leung, N.Y.H., Wai, C.Y.Y., Shu, S.A., Chang, C.C., Chu, K.H., Leung, P.S.C., 2017. Low-dose allergen-specific immunotherapy induces tolerance in a murine model of shrimp allergy. *Int. Arch. Allergy Immunol.* 174 (2), 86–96.
- Leung, P.S., Chen, Y.C., Gershwin, M.E., Wong, S.H., Kwan, H.S., Chu, K.H., 1998a. Identification and molecular characterization of *Charybdis feriatus* tropomyosin, the major crab allergen. *J. Allergy Clin. Immunol.* 102 (5), 847–852.
- Leung, P.S., Chu, K.H., 2001. cDNA cloning and molecular identification of the major oyster allergen from the Pacific oyster *Crassostrea gigas*. *Clin. Exp. Allergy* 31 (8), 1287–1294.
- Leung, P.S., Chu, K.H., Chow, W.K., Ansari, A., Bandea, C.I., Kwan, H.S., Nagy, S.M., Gershwin, M.E., 1994. Cloning, expression, and primary structure of *Metapenaeus ensis* tropomyosin, the major heat-stable shrimp allergen. *J. Allergy Clin. Immunol.* 94 (5), 882–890.
- Leung, P.S., Lee, Y.S., Tang, C.Y., Kung, W.Y., Chuang, Y.H., Chiang, B.L., Fung, M.C., Chu, K.H., 2008. Induction of shrimp tropomyosin-specific hypersensitivity in mice. *Int. Arch. Allergy Immunol.* 147 (4), 305–314.
- Leung, P.S.C., Chen, Y.C., Mykles, D.L., Chow, W.K., Li, C.P., Chu, K.H., 1998b. Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. *Mol. Mar. Biol. Biotech.* 7 (1), 12–20.
- Leung, P.S.C., Chow, W.K., Duffey, S., Kwan, H.S., Gershwin, M.E., Chu, K.H., 1996. IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. *J. Allergy Clin. Immun.* 98 (5), 954–961.
- Li, Z., You, J., Luo, Y., Wu, J., 2014. Purification and characterization of parvalbumin isoforms from grass carp (*Ctenopharyngodon idella*). *J. Agric. Food Chem.* 62 (26), 6212–6218.
- Liang, J., Tan, C.C., Taylor, S.L., Baumert, J.L., Lopata, A.L., Lee, N.A., 2017. Quantitative analysis of species specificity of two anti-parvalbumin antibodies for detecting southern hemisphere fish species demonstrating strong phylogenetic association. *Food Chem.* 237, 588–596.
- Liang, Y.L., Cao, M.J., Su, W.J., Zhang, L.J., Huang, Y.Y., Liu, G.M., 2008. Identification and characterization of the major allergen of Chinese mitten crab (*Eriocheir sinensis*). *Food Chem.* 111 (4), 998–1003.
- Lim, D.L., Neo, K.H., Goh, D.L., Shek, L.P., Lee, B.W., 2005. Missing parvalbumin: implications in diagnostic testing for tuna allergy. *J. Allergy Clin. Immunol.* 115 (4), 874–875.
- Lim, D.L., Neo, K.H., Yi, F.C., Chua, K.Y., Goh, D.L., Shek, L.P., Giam, Y.C., Van Bever, H.P., Lee, B.W., 2008. Parvalbumin—the major tropical fish allergen. *Pediatr. Allergy Immunol.* 19 (5), 399–407.
- Lindstrom, C.D., van Do, T., Hordvik, I., Endresen, C., Elsayed, S., 1996. Cloning of two distinct cDNAs encoding parvalbumin, the major allergen of Atlantic Salmon (*Salmo salar*). *Scand. J. Immunol.* 44 (4), 335–344.
- Liu, G.M., Huang, Y.Y., Cai, Q.F., Weng, W.Y., Su, W.J., Cao, M.J., 2011a. Comparative study of in vitro digestibility of major allergen, tropomyosin and other proteins between Grass prawn (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*). *J. Sci. Food Agric.* 91 (1), 163–170.
- Liu, R., Holck, A.L., Yang, E., Liu, C., Xue, W., 2013. Tropomyosin from tilapia (*Oreochromis mossambicus*) as an allergen. *Clin. Exp. Allergy* 43 (3), 365–377.
- Liu, R., Krishnan, H.B., Xue, W., Liu, C., 2011b. Characterization of allergens isolated from the freshwater fish blunt snout bream (*Megalobrama amblycephala*). *J. Agric. Food Chem.* 59 (1), 458–463.
- Liu, R., Yang, E., Liu, C., Xue, W., 2012. Tilapia (*Oreochromis mossambicus*) allergens characterized by ELISA, SDS-PAGE, 2D gels, Western blotting and MALDI-TOF mass spectrometry. *Int. J. Food Sci. Nutr.* 63 (3), 259–266.
- Liu, Y.Y., Chen, X.F., Hu, J.W., Chen, Z.W., Zhang, L.J., Cao, M.J., Liu, G.M., 2016. Purification and characterization of protamine, the allergen from the milt of large yellow croaker (*Pseudosciaena crocea*), and its components. *J. Agric. Food Chem.* 64 (9), 1999–2011.
- Liu, Z., Xia, L., Wu, Y., Xia, Q., Chen, J., Roux, K.H., 2009. Identification and characterization of an arginine kinase as a major allergen from silkworm (*Bombyx mori*) larvae. *Int. Arch. Allergy Immunol.* 150 (1), 8–14.
- Lopata, A.L., Jeebhay, M.F., 2013. Airborne seafood allergens as a cause of occupational allergy and asthma. *Curr. Allergy Asthma Rep.* 13 (3), 288–297.
- Lopata, A.L., Jeebhay, M.F., Reese, G., Fernandes, J., Swoboda, I., Robins, T.G., Lehrer, S.B., 2005. Detection of fish antigens aerosolized during fish processing using newly developed immunoassays. *Int. Arch. Allergy Immunol.* 138 (1), 21–28.
- Lopata, A.L., Kamath, S., 2012. Shellfish allergy diagnosis - gaps and needs. *Curr. Allergy Clin. Immunol.* 25 (2), 60–66.
- Lopata, A.L., Kleine-Tebbe, J., Kamath, S.D., 2016. Allergens and molecular diagnostics of shellfish allergy: part 22 of the series molecular allergology. *Allergo. J. Int.* 25 (7), 210–218.
- Lopata, A.L., Zinn, C., Potter, P.C., 1997. Characteristics of hypersensitivity reactions and identification of a unique 49 kd IgE-binding protein (Hal-m-1) in abalone (*Haliotis midae*). *J. Allergy Clin. Immunol.* 100 (5), 642–648.
- Lopez-Matas, M.A., de Larramendi, C.H., Moya, R., Sanchez-Guerrero, I., Ferrer, A., Huertas, A.J., Flores, I., Navarro, L.A., Garcia-Abujeta, J.L., Vicario, S., Andreu, C., Pena, M., Carnes, J., 2016. In vivo diagnosis with purified tropomyosin in mite and shellfish allergic patients. *Ann. Allergy Asthma Immunol.* 116 (6), 538–543.
- Lopez-Zavala, A.A., Carrasco-Miranda, J.S., Ramirez-Aguirre, C.D., Lopez-Hidalgo, M., Benitez-Cardoza, C.G., Ochoa-Leyva, A., Cardona-Felix, C.S., Diaz-Quezada, C., Rudino-Pinera, E., Sotelo-Mundo, R.R., Brieba, L.G., 2016. Structural insights from a novel invertebrate triosephosphate isomerase from *Litopenaeus vannamei*. *Biochim. Biophys. Acta* 1864 (12), 1696–1706.
- Lopez-Zavala, A.A., Garcia-Orozco, K.D., Carrasco-Miranda, J.S., Sugich-Miranda, R., Velazquez-Contreras, E.F., Criscitiello, M.F., Brieba, L.G., Rudino-Pinera, E., Sotelo-Mundo, R.R., 2013. Crystal structure of shrimp arginine kinase in binary complex with arginine—a molecular view of the phosphagen precursor binding to the enzyme. *J. Bioenerg. Biomembr.* 45 (6), 511–518.
- Lopez-Zavala, A.A., Sotelo-Mundo, R.R., Garcia-Orozco, K.D., Isac-Martinez, F., Brieba, L.G., Rudino-Pinera, E., 2012. Crystallization and X-ray diffraction studies of arginine kinase from the white Pacific shrimp *Litopenaeus vannamei*. *Acta Crystallogr. F-Struct. Biol. Cryst. Commun.* 68 (Pt. 7), 783–785.
- Ma, Y., Griesmeier, U., Susani, M., Radauer, C., Briza, P., Erler, A., Bublin, M., Alessandri, S., Himly, M., Vazquez-Cortes, S., de Arellano, I.R., Vassilopoulou, E., Saxoni-Papageorgiou, P., Knulst, A.C., Fernandez-Rivas, M., Hoffmann-Sommergruber, K., Breiteneder, H., 2008. Comparison of natural and recombinant forms of the major fish allergen parvalbumin from cod and carp. *Mol. Nutr. Food Res.* 52 (Suppl 2), S196–S207.
- Mahajan, A., Youssef, L.A., Cleary, C., Grattan, R., Lucero, S.R., Mattison, C.P., Erasmus, M.F., Jacobson, B., Tapia, L., Hlavacek, W.S., Schuyler, M., Wilson, S., 2017. Allergen valency, dose, and fcepsilonRI occupancy set thresholds for secretory responses to Pen a 1 and motivate design of hypoallergens. *J. Immunol.* 198 (3), 1034–1046.
- Mäkinen-Kiljunen, S., Kiistala, R., Varjonen, E., 2003. Severe reactions from roe without concomitant fish allergy. *Ann. Allergy Asthma Immunol.* 91 (4), 413–416.
- Martin-Garcia, C., Carnes, J., Blanco, R., Martinez-Alonso, J.C., Callejo-Melgosa, A., Frades, A., Colino, T., 2007. Selective hypersensitivity to boiled razor shell. *J. Invest. Allergol. Clin. Immunol.* 17 (4), 271–273.
- Matricardi, P.M., Kleine-Tebbe, J., Hoffmann, H.J., Valenta, R., Hilger, C., Hofmaier, S., Aalberse, R.C., Agache, I., Asero, R., Ballmer-Weber, B., Barber, D., Beyer, K., Biedermann, T., Bilo, M.B., Blank, S., Bohle, B., Bosshard, P.P., Breiteneder, H., Brough, H.A., Caraballo, L., Caubet, J.C., Cramer, R., Davies, J.M., Douladiris, N., Ebisawa, M., Pa, E.I., Fernandez-Rivas, M., Ferreira, F., Gadermaier, G., Glatz, M., Hamilton, R.G., Hawranek, T., Helling, P., Hoffmann-Sommergruber, K., Jakob, T., Jappe, U., Jutel, M., Kamath, S.D., Knol, E.F., Korosec, P., Kuehn, A., Lack, G., Lopata, A.L., Makela, M., Morisset, M., Niederberger, V., Nowak-Wegrzyn, A.H., Papadopoulos, N.G., Pastorello, E.A., Pauli, G., Platts-Mills, T., Posa, D., Poulsen, L.K., Raulf, M., Sastre, J., Scala, E., Schmid, J.M., Schmid-Grendelmeier, P., van Hage, M., van Ree, R., Vieths, S., Weber, R., Wickman, M., Muraro, A., Ollert, M., 2016. EAACI molecular allergology user's guide. *Pediatr. Allergy Immunol.* 27 (Suppl 23), 1–250.
- McGowan, E.C., Keet, C.A., 2013. Prevalence of self-reported food allergy in the National Health and Nutrition Examination Survey (NHANES) 2007–2010. *J. Allergy Clin. Immunol.* 132 (5), 1216–1219 e1215.
- Merkulova, T., Lucas, M., Jabet, C., Lamande, N., Rouzeau, J.D., Gros, F., Lazar, M., Keller, A., 1997. Biochemical characterization of the mouse muscle-specific enolase: developmental changes in electrophoretic variants and selective binding to other proteins. *Biochem. J.* 323 (Pt. 3), 791–800.
- Mita, H., Koketsu, A., Ishizaki, S., Shiomi, K., 2013. Molecular cloning and functional expression of allergenic sarcoplasmic calcium-binding proteins from *Penaeus* shrimps. *J. Sci. Food Agric.* 93 (7), 1737–1742.
- Miyazawa, H., Fukamachi, H., Inagaki, Y., Reese, G., Daul, C.B., Lehrer, S.B., Inouye, S., Sakaguchi, M., 1996. Identification of the first major allergen of a squid (*Todarodes pacificus*). *J. Allergy Clin. Immunol.* 98 (Pt. 5), 948–953.
- Mohammadi, M., Mokhtarian, K., Kardar, G.A., Farokhi, S., Sadroddini, E., Khorramizadeh, M.R., Falak, R., 2017. Expression of recombinant parvalbumin from wolf-herring fish and determination of its IgE-binding capability. *Food Agric. Immunol.* 28 (4), 573–585.
- Moonesinghe, H., Mackenzie, H., Venter, C., Kilburn, S., Turner, P., Weir, K., Dean, T., 2016. Prevalence of fish and shellfish allergy: a systematic review. *Ann. Allergy Asthma Immunol.* 117 (3), 264–272 e264.
- Moraes, A.H., Ackerbauer, D., Kostadinova, M., Bublin, M., de Oliveira, G.A., Ferreira, F., Almeida, F.C., Breiteneder, H., Valente, A.P., 2014. Solution and high-pressure NMR studies of the structure, dynamics, and stability of the cross-reactive allergenic cod parvalbumin Gad m 1. *Proteins* 82 (11), 3032–3042.
- Moreno, F.J., 2007. Gastrointestinal digestion of food allergens: effect on their allergenicity. *Biomed. Pharmacother.* 61 (1), 50–60.
- Mori, A., Mita, H., Ishizaki, S., Shiomi, K., 2013. Importance of conformation for the IgE reactivity of sarcoplasmic calcium-binding protein from the black tiger shrimp *Penaeus monodon*. *Eur. Food Res. Technol.* 236 (1), 165–170.
- Motoyama, K., Ishizaki, S., Nagashima, Y., Shiomi, K., 2006. Cephalopod tropomyosins: identification as major allergens and molecular cloning. *Food Chem. Toxicol.* 44 (12), 1997–2002.
- Motoyama, K., Suma, Y., Ishizaki, S., Nagashima, Y., Lu, Y., Ushio, H., Shiomi, K., 2008. Identification of tropomyosins as major allergens in antarctic krill and mantis shrimp and their amino acid sequence characteristics. *Mar. Biotechnol. (NY)* 10 (6), 709–718.
- Motoyama, K., Suma, Y., Ishizaki, S., Nagashima, Y., Shiomi, K., 2007. Molecular cloning of tropomyosins identified as allergens in six species of crustaceans. *J. Agric. Food Chem.* 55 (3), 985–991.
- Mykles, D.L., Cotton, J.L.S., Taniguchi, H., Sano, K.I., Maeda, Y., 1998. Cloning of tropomyosins from lobster (*Homarus americanus*) striated muscles: fast and slow isoforms may be generated from the same transcript. *J. Muscle Res. Cell Motil.* 19 (2),

- 105–115.
- Myrset, H.R., Barletta, B., Di Felice, G., Egaas, E., Dooper, M.M., 2013. Structural and immunological characterization of recombinant Pan b 1, a major allergen of northern shrimp, *Pandalus borealis*. *Int. Arch. Allergy Immunol.* 160 (3), 221–232.
- Nakamura, A., Sasaki, F., Watanabe, K., Ojima, T., Ahn, D.H., Saeki, H., 2006. Changes in allergenicity and digestibility of squid tropomyosin during the Maillard reaction with ribose. *J. Agric. Food Chem.* 54 (25), 9529–9534.
- Nakamura, A., Watanabe, K., Ojima, T., Ahn, D.H., Saeki, H., 2005. Effect of maillard reaction on allergenicity of scallop tropomyosin. *J. Agric. Food Chem.* 53 (19), 7559–7564.
- Nakamura, R., Satoh, R., Nakajima, Y., Kawasaki, N., Yamaguchi, T., Sawada, J., Nagoya, H., Teshima, R., 2009. Comparative study of GH-transgenic and non-transgenic amago salmon (*Oncorhynchus masou ishikawae*) allergenicity and proteomic analysis of amago salmon allergens. *Regul. Toxicol. Pharmacol.* 55 (3), 300–308.
- Nakano, S., Yoshinuma, T., Yamada, T., 2008. Reactivity of shrimp allergy-related IgE antibodies to krill tropomyosin. *Int. Arch. Allergy Immunol.* 145 (3), 175–181.
- Nezvorov, I.A., Levitsky, D.I., 2011. Tropomyosin: double helix from the protein world. *Biochem.-Moscow* 76 (13), 1507–1527.
- Nieuwenhuizen, N., Lopata, A.L., Jeebhay, M.F., Herbert, D.R., Robins, T.G., Brombacher, F., 2006. Exposure to the fish parasite *Anisakis* causes allergic airway hyperreactivity and dermatitis. *J. Allergy Clin. Immunol.* 117 (5), 1098–1105.
- Nieuwenhuizen, N.E., Lopata, A.L., 2013. *Anisakis*—a food-borne parasite that triggers allergic host defences. *Int. J. Parasitol.* 43 (12–13), 1047–1057.
- Nieuwenhuizen, N.E., Lopata, A.L., 2014. Allergic reactions to *Anisakis* found in fish. *Curr. Allergy Asthma Rep.* 14 (8), 455.
- Niu, X., Bruschweiler-Li, L., Davulcu, O., Skaliky, J.J., Bruschweiler, R., Chapman, M.S., 2011. Arginine kinase: joint crystallographic and NMR RDC analyses link substrate-associated motions to intrinsic flexibility. *J. Mol. Biol.* 405 (2), 479–496.
- Nugraha, R., Kamath, S.D., Johnston, E., Zenger, K.R., Rolland, J.M., O'Hehir, R.E., Lopata, A.L., 2018. Rapid and comprehensive discovery of unreported shellfish allergens using large-scale transcriptomic and proteomic resources. *J. Allergy Clin. Immunol.* 141 (4), 1501–1504 e1508.
- Oguchi, Y., Ishizuka, J., Hitchcock-DeGregori, S.E., Ishiwata, S., Kawai, M., 2011. The role of tropomyosin domains in cooperative activation of the actin-myosin interaction. *J. Mol. Biol.* 414 (5), 667–680.
- Ojima, T., Toyoguchi, T., Nishita, K., 1995. Isolation and characterization of tropomyosin from abdominal muscle of prawn *Penaeus japonicus*. *Fish. Sci.* 61 (5), 871–875.
- Ostblom, E., Wickman, M., van Hage, M., Lilja, G., 2008. Reported symptoms of food hypersensitivity and sensitization to common foods in 4-year-old children. *Acta Paediatr.* 97 (1), 85–90.
- Osterballe, M., Mortz, C.G., Hansen, T.K., Andersen, K.E., Bindslev-Jensen, C., 2009. The prevalence of food hypersensitivity in young adults. *Pediatr. Allergy Immunol.* 20 (7), 686–692.
- Pan, B.Q., Su, W.J., Cao, M.J., Cai, Q.F., Weng, W.Y., Liu, G.M., 2012. IgE reactivity to type I collagen and its subunits from *Tilapia* (*Tilapia zillii*). *Food Chem.* 130 (1), 127–133.
- Pascal, M., Grishina, G., Yang, A.C., Sanchez-Garcia, S., Lin, J., Towle, D., Ibanez, M.D., Sastre, J., Sampson, H.A., Ayuso, R., 2015. Molecular diagnosis of shrimp allergy: efficiency of several allergens to predict clinical reactivity. *J. Allergy Clin. Immunol. Pract.* 3 (4), 521–529 e510.
- Pechere, J.F., Demaille, J., Capony, J.P., 1971. Muscular parvalbumins: preparative and analytical methods of general applicability. *Biochim. Biophys. Acta* 236 (2), 391–408.
- Perez-Gordo, M., Cuesta-Herranz, J., Maroto, A.S., Cases, B., Ibanez, M.D., Vivanco, F., Pastor-Vargas, C., 2011. Identification of sole parvalbumin as a major allergen: study of cross-reactivity between parvalbumins in a Spanish fish-allergic population. *Clin. Exp. Allergy* 41 (5), 750–758.
- Perez-Gordo, M., Lin, J., Bardina, L., Pastor-Vargas, C., Cases, B., Vivanco, F., Cuesta-Herranz, J., Sampson, H.A., 2012. Epitope mapping of Atlantic salmon major allergen by peptide microarray immunoassay. *Int. Arch. Allergy Immunol.* 157 (1), 31–40.
- Perez-Gordo, M., Pastor-Vargas, C., Lin, J., Bardina, L., Cases, B., Ibanez, M.D., Vivanco, F., Cuesta-Herranz, J., Sampson, H.A., 2013. Epitope mapping of the major allergen from Atlantic cod in Spanish population reveals different IgE-binding patterns. *Mol. Nutr. Food Res.* 57 (7), 1283–1290.
- Perez-Gordo, M., Sanchez-Garcia, S., Cases, B., Pastor, C., Vivanco, F., Cuesta-Herranz, J., 2008. Identification of vitellogenin as an allergen in Beluga caviar allergy. *Allergy* 63 (4), 479–480.
- Perez-Perez, J., Fernandez-Caldas, E., Maranon, F., Sastre, J., Bernal, M.L., Rodriguez, J., Bedate, C.A., 2000. Molecular cloning of paramyosin, a new allergen of *Anisakis* simplex. *Int. Arch. Allergy Immunol.* 123 (2), 120–129.
- Permyakov, S.E., Vologzhannikova, A.A., Emelyanenko, V.I., Knyazeva, E.L., Kazakov, A.S., Lapteva, Y.S., Permyakova, M.E., Zhadan, A.P., Permyakov, E.A., 2012. The impact of alpha-N-acetylation on structural and functional status of parvalbumin. *Cell Calcium* 52 (5), 366–376.
- Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* 25 (13), 1605–1612.
- Poore, G., 2004. Marine Decapod Crustacea of Southern Australia: A Guide to Identification. Melbourne.
- Pruett, P.S., Azzi, A., Clark, S.A., Yousef, M.S., Gattis, J.L., Somasundaram, T., Ellington, W.R., Chapman, M.S., 2003. The putative catalytic bases have, at most, an accessory role in the mechanism of arginine kinase. *J. Biol. Chem.* 278 (29), 26952–26957.
- Pyrhonen, K., Nayha, S., Kaila, M., Hiltunen, L., Laara, E., 2009. Occurrence of parent-reported food hypersensitivities and food allergies among children aged 1–4 yr. *Pediatr. Allergy Immunol.* 20 (4), 328–338.
- Radauer, C., Bublin, M., Wagner, S., Mari, A., Breiteneder, H., 2008. Allergens are distributed into few protein families and possess a restricted number of biochemical functions. *J. Allergy Clin. Immunol.* 121 (4), 847–852 e847.
- Rance, F., Grandmottet, X., Grandjean, H., 2005. Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. *Clin. Exp. Allergy* 35 (2), 167–172.
- Reddy, S.R., Houmeida, A., Benyamin, Y., Roustan, C., 1992. Interaction in vitro of scallop muscle arginine kinase with filamentous actin. *Eur. J. Biochem.* 206 (1), 251–257.
- Reese, G., Ayuso, R., Lehrer, S.B., 1999. Tropomyosin: an invertebrate Pan-Allergen. *Int. Arch. Allergy Immunol.* 119 (4), 247–258.
- Reese, G., Viebranz, J., Leong-Kee, S.M., Plante, M., Lauer, I., Randow, S., Moncin, M.S., Ayuso, R., Lehrer, S.B., Vieths, S., 2005. Reduced allergenic potency of VR9-1, a mutant of the major shrimp allergen Pen a 1 (tropomyosin). *J. Immunol.* 175 (12), 8354–8364.
- Richardson, R.C., King, N.M., Harrington, D.J., Sun, H., Royer, W.E., Nelson, D.J., 2000. X-Ray crystal structure and molecular dynamics simulations of silver hake parvalbumin (Isoform B). *Protein Sci.* 9 (1), 73–82.
- Rodriguez-Del Rio, P., Sanchez-Lopez, J., Robledo Echarren, T., Martinez-Cocera, C., Fernandez-Rivas, M., 2009. Selective allergy to *Venus antiqua* clam. *Allergy* 64 (5), 815.
- Rohrbach, S.E., Wheatly, M.G., Gillen, C.M., 2015. Calcium binding to Procambarus clarkii sarcoplasmic calcium binding protein splice variants. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 179, 57–63.
- Roquet, F., Declercq, J.P., Tinant, B., Rambaud, J., Parello, J., 1992. Crystal structure of the unique parvalbumin component from muscle of the leopard shark (Triakis semifasciata). The first X-ray study of an alpha-parvalbumin. *J. Mol. Biol.* 223 (3), 705–720.
- Rosmilah, M., Shahnaz, M., Masita, A., Noormalin, A., Jamaludin, M., 2005. Identification of major allergens of two species of local snappers: *Lutjanus argentimaculatus* (merah/ red snapper) and *Lutjanus johnii* (jenahak/ golden snapper). *Trop. Biomed.* 22 (2), 171–177.
- Rouvinen, J., Janis, J., Laukkanen, M.L., Jylha, S., Niemi, M., Paivinen, T., Makinen-Kiljunen, S., Hahtela, T., Soderlund, H., Takkinen, K., 2010. Transient dimers of allergens. *PLoS One* 5 (2) e9037.
- Ruethers, T., Raith, M., Sharp, M.F., Koerber, M., Stephen, J.N., Nugraha, R., Le, T.T.K., Quirce, S., Nguyen, H.X.M., Kamath, S.D., Mehr, S.S., Campbell, D.E., Bridges, C.R., Taki, A.C., Swoboda, I., Lopata, A.L., 2018. Characterization 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* 48 (4), 452–463.
- Sakaguchi, M., Toda, M., Ebihara, T., Irie, S., Hori, H., Imai, A., Yanagida, M., Miyazawa, H., Ohsumi, H., Ikezawa, Z., Inouye, S., 2000. IgE antibody to fish gelatin (type I collagen) in patients with fish allergy. *J. Allergy Clin. Immunol.* 106 (3), 579–584.
- Sanchez, R., Martinez, J., Castro, A., Pedrosa, M., Quirce, S., Rodriguez-Perez, R., Gasset, M., 2016. The amyloid fold of Gad m 1 epitopes governs IgE binding. *Sci. Rep.* 6, 32801.
- Saptarshi, S.R., Sharp, M.F., Kamath, S.D., Lopata, A.L., 2014. Antibody reactivity to the major fish allergen parvalbumin is determined by isoforms and impact of thermal processing. *Food Chem.* 148, 321–328.
- Schevzov, G., Whittaker, S.P., Fath, T., Lin, J.J., Gunning, P.W., 2011. Tropomyosin isoforms and reagents. *Bioarchitecture* 1 (4), 135–164.
- Schiavi, E., Barletta, B., Butteroni, C., Corinti, S., Boirivant, M., Felice, G.D., 2011. Oral therapeutic administration of a probiotic mixture suppresses established Th2 responses and systemic anaphylaxis in a murine model of food allergy. *Allergy* 66 (4), 499–508.
- Schnabel, E., Sausenthaler, S., Schaaf, B., Schafer, T., Lehmann, I., Behrendt, H., Herbarth, O., Borte, M., Kramer, U., von Berg, A., Wichmann, H.E., Heinrich, J., Group, L.S., 2010. Prospective association between food sensitization and food allergy: results of the LISA birth cohort study. *Clin. Exp. Allergy* 40 (3), 450–457.
- Shakib, F., Ghaemmaghami, A.M., Sewell, H.F., 2008. The molecular basis of allergenicity. *Trends Immunol.* 29 (12), 633–642.
- Shanti, K.N., Martin, B.M., Nagpal, S., Metcalfe, D.D., Rao, P.V.S., 1993. Identification of tropomyosin as the major shrimp allergen and characterization of its ige-binding epitopes. *J. Immunol.* 151 (10), 5354–5363.
- Sharp, M.F., Kamath, S.D., Koerber, M., Jerry, D.R., O'Hehir, R.E., Campbell, D.E., Lopata, A.L., 2014. Differential IgE binding to isoallergens from Asian seabass (*Lates calcarifer*) in children and adults. *Mol. Immunol.* 62 (1), 77–85.
- Sharp, M.F., Lopata, A.L., 2014. Fish allergy: in review. *Clin. Rev. Allergy Immunol.* 46 (3), 258–271.
- Sharp, M.F., Stephen, J.N., Kraft, L., Weiss, T., Kamath, S.D., Lopata, A.L., 2015. Immunological cross-reactivity between four distant parvalbumins-Impact on allergen detection and diagnostics. *Mol. Immunol.* 63 (2), 437–448.
- Shen, H.W., Cao, M.J., Cai, Q.F., Ruan, M.M., Mao, H.Y., Su, W.J., Liu, G.M., 2012. Purification, cloning, and immunological characterization of arginine kinase, a novel allergen of Octopus fangsiao. *J. Agric. Food Chem.* 60 (9), 2190–2199.
- Shen, Y., Cao, M.J., Cai, Q.F., Su, W.J., Yu, H.L., Ruan, W.W., Liu, G.M., 2011. Purification, cloning, expression and immunological analysis of *Scylla serrata* arginine kinase, the crab allergen. *J. Sci. Food Agric.* 91 (7), 1326–1335.
- Shimizu, Y., Kishimura, H., Kanno, G., Nakamura, A., Adachi, R., Akiyama, H., Watanabe, K., Hara, A., Ebisawa, M., Saeki, H., 2014. Molecular and immunological characterization of beta'-component (Onc k 5), a major IgE-binding protein in chum salmon roe. *Int. Immunol.* 26 (3), 139–147.
- Shimizu, Y., Nakamura, A., Kishimura, H., Hara, A., Watanabe, K., Saeki, H., 2009. Major allergen and its IgE cross-reactivity among salmonid fish roe allergy. *J. Agric. Food Chem.* 57 (6), 2314–2319.
- Shiomi, K., Sato, Y., Hamamoto, S., Mita, H., Shimakura, K., 2008. Sarcoplasmic calcium-binding protein: identification as a new allergen of the black tiger shrimp *Penaeus*

- monodon. *Int. Arch. Allergy Immunol.* 146 (2), 91–98.
- Sicherer, S.H., Sampson, H.A., 2010. Food allergy. *J. Allergy Clin. Immunol.* 125 (2), S116–S125.
- Song, J., Li, L., Liu, Z., Li, Q., Ran, P., 2009. Sequence analysis and expression of a cDNA clone encoding tropomyosin in *Sinonovacula constricta*. *Mol. Biol. Rep.* 36 (2), 315–321.
- Sorensen, M., Kuehn, A., Mills, E.N.C., Costello, C.A., Ollert, M., Smabrekke, L., Primicerio, R., Wickman, M., Klingenberg, C., 2017. Cross-reactivity in fish allergy: a double-blind, placebo-controlled food-challenge trial. *J. Allergy Clin. Immunol.* 140 (4), 1170–1172.
- Srinroch, C., Srisomsap, C., Chokchaichamnankit, D., Punyari, P., Phiriyangkul, P., 2015. Identification of novel allergen in edible insect, *Gryllus bimaculatus* and its cross-reactivity with *Macrobrachium* spp. allergens. *Food Chem.* 184, 160–166.
- Srivastava, K.D., Bardina, L., Sampson, H.A., Li, X.M., 2012. Efficacy and immunological actions of FAHF-2 in a murine model of multiple food allergies. *Ann. Allergy Asthma Immunol.* 108 (5), 351–358 e351.
- Staudacher, E., Stepan, H., Gutterigg, M., 2009. Protein N-Glycosylation of gastropods. *Curr. Top. Biochem. Res.* 11 (2), 29–39.
- Stephen, J., Sharp, M., Ruethers, T., Taki, A., Campbell, D., Lopata, A., 2017. Allergenicity of bony and cartilaginous fish—molecular and immunological properties. *Clin. Exp. Allergy* 47 (3), 300–312.
- Subba Rao, P.V., Rajagopal, D., Ganesh, K.A., 1998. B- and T-cell epitopes of tropomyosin, the major shrimp allergen. *Allergy* 53 (Suppl. (46)), 44–47.
- Sulbaran, G., Alamo, L., Pinto, A., Marquez, G., Mendez, F., Padron, R., Craig, R., 2015. An invertebrate smooth muscle with striated muscle myosin filaments. *Proc. Natl. Acad. Sci. U. S. A.* 112 (42), E5660–E5668.
- Suzuki, M., Kobayashi, Y., Hiraki, Y., Nakata, H., Shiomi, K., 2011. Paramyosin of the disc abalone *Haliotis discus discus*: identification as a new allergen and cross-reactivity with tropomyosin. *Food Chem.* 124 (3), 921–926.
- Suzuki, M., Shimizu, K., Kobayashi, Y., Ishizaki, S., Shiomi, K., 2014. Paramyosin from the disc abalone *Haliotis discus discus*. *J. Food Biochem.* 38 (4), 444–451.
- Swain, A.L., Kretsinger, R.H., Amma, E.L., 1989. Restrained least squares refinement of native (calcium) and cadmium-substituted carp parvalbumin using X-ray crystallographic data at 1.6-Å resolution. *J. Biol. Chem.* 264 (28), 16620–16628.
- Swoboda, I., Balic, N., Klug, C., Focke, M., Weber, M., Spitzauer, S., Neubauer, A., Quirce, S., Douladiris, N., Papadopoulos, N.G., Valenta, R., 2013. A general strategy for the generation of hypoallergenic molecules for the immunotherapy of fish allergy. *J. Allergy Clin. Immunol.* 132 (4), 979–981 e971.
- Swoboda, I., Bugajska-Schretter, A., Linhart, B., Verdino, P., Keller, W., Schulmeister, U., Sperr, W.R., Valent, P., Peltre, G., Quirce, S., Douladiris, N., Papadopoulos, N.G., Valenta, R., Spitzauer, S., 2007. A recombinant hypoallergenic parvalbumin mutant for immunotherapy of IgE-mediated fish allergy. *J. Immunol.* 178 (10), 6290–6296.
- Swoboda, I., Bugajska-Schretter, A., Valenta, R., Spitzauer, S., 2002a. Recombinant fish parvalbumins: candidates for diagnosis and treatment of fish allergy. *Allergy* 57 (Suppl. 72), 94–96.
- Swoboda, I., Bugajska-Schretter, A., Verdino, P., Keller, W., Sperr, W.R., Valent, P., Valenta, R., Spitzauer, S., 2002b. Recombinant carp parvalbumin, the major cross-reactive fish allergen: a tool for diagnosis and therapy of fish allergy. *J. Immunol.* 168 (9), 4576–4584.
- Takagi, T., Kobayashi, T., Konishi, K., 1984. Amino-Acid-Sequence of sarcoplasmic calcium-binding protein from scallop (*Patinopekten-Yessoensis*) Adductor Striated-Muscle. *Biochim. Biophys. Acta* 787 (3), 252–257.
- Tanaka, H., Takahashi, H., Ojima, T., 2013. Ca(2+) + -binding properties and regulatory roles of lobster troponin C sites II and IV. *FEBS Lett.* 587 (16), 2612–2616.
- Thomassen, M.R., Kamath, S.D., Lopata, A.L., Madsen, A.M., Eduard, W., Bang, B.E., Aasmoe, L., 2016. Occupational exposure to bioaerosols in Norwegian crab processing plants. *Ann. Occup. Hyg.* 60 (7), 781–794.
- Trevino, S., 1998. Fish and shellfish poisoning. *Clin. Lab. Sci.* 11 (5), 309–314.
- Uda, K., Fujimoto, N., Akiyama, Y., Mizuta, K., Tanaka, K., Ellington, W.R., Suzuki, T., 2006. Evolution of the arginine kinase gene family. *Comp. Biochem. Physiol. Part D Genom. Proteom.* 1 (2), 209–218.
- Untersmayr, E., Szalai, K., Riener, A.B., Hemmer, W., Swoboda, I., Hantusch, B., Scholl, I., Spitzauer, S., Scheiner, O., Jarisch, R., Boltz-Nitulescu, G., Jensen-Jarolim, E., 2006. Mimotopes identify conformational epitopes on parvalbumin, the major fish allergen. *Mol. Immunol.* 43 (9), 1454–1461.
- Usui, M., Harada, A., Ishimaru, T., Sakumichi, E., Saratani, F., Sato-Minami, C., Azakami, H., Miyasaki, T., Hanaoka, K., 2013. Contribution of structural reversibility to the heat stability of the tropomyosin shrimp allergen. *Biosci. Biotech. Biochem.* 77 (5), 948–953.
- van der Ventel, M.L., Nieuwenhuizen, N.E., Kirstein, F., Hikuam, C., Jeebhay, M.F., Swoboda, I., Brombacher, F., Lopata, A.L., 2011. Differential responses to natural and recombinant allergens in a murine model of fish allergy. *Mol. Immunol.* 48 (4), 637–646.
- Van Do, T., Elsayed, S., Florvaag, E., Hordvik, I., Endresen, C., 2005. Allergy to fish parvalbumins: studies on the cross-reactivity of allergens from 9 commonly consumed fish. *J. Allergy Clin. Immunol.* 116 (6), 1314–1320.
- 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*). *Scand. J. Immunol.* 50 (6), 619–625.
- Van Do, T., Hordvik, I., Endresen, C., Elsayed, S., 2003. The major allergen (parvalbumin) of codfish is encoded by at least two isotypic genes: cDNA cloning, expression and antibody binding of the recombinant allergens. *Mol. Immunol.* 39 (10), 595–602.
- Viscaino, P., Schirone, M., Berti, M., Milandri, A., Tofalo, R., Suzzi, G., 2016. Marine biotoxins: occurrence, toxicity, regulatory limits and reference methods. *Front. Microbiol.* 7, 1051.
- Volbeda, A., Hol, W.G.J., 1989. Crystal structure of hexameric haemocyanin from *Panulirus interruptus* refined at 3.2 Å resolution. *J. Mol. Biol.* 209 (2), 249–279.
- Vologzhannikova, A.A., Khorn, P.A., Kazakov, A.S., Ismailov, R.G., Sokolov, A.S., Uversky, V.N., Permyakov, E.A., Permyakov, S.E., 2017. In search for globally disordered apo-parvalbumins: case of parvalbumin beta-1 from coho salmon. *Cell Calcium* 67, 53–64.
- Wai, C.Y., Leung, N.Y., Ho, M.H., Gershwin, L.J., Shu, S.A., Leung, P.S., Chu, K.H., 2014. Immunization with Hypoallergens of shrimp allergen tropomyosin inhibits shrimp tropomyosin specific IgE reactivity. *PLoS One* 9 (11), e11649.
- Wai, C.Y., Leung, N.Y., Leung, P.S., Chu, K.H., 2016. T cell epitope immunotherapy ameliorates allergic responses in a murine model of shrimp allergy. *Clin. Exp. Allergy* 46 (3), 491–503.
- Wang, B., Li, Z., Zheng, L., Liu, Y., Lin, H., 2011a. Identification and characterization of a new IgE-binding protein in mackerel (*Scomber japonicus*) by MALDI-TOF-MS. *J. Ocean Univ. China* 10 (1), 93–98.
- Wang, J., Calatroni, A., Visness, C.M., Sampson, H.A., 2011b. Correlation of specific IgE to shrimp with cockroach and dust mite exposure and sensitization in an inner-city population. *J. Allergy Clin. Immunol.* 128 (4), 834–837.
- Wang, J.S., Zheng, Z.L., Lei, J., Pan, J.C., Zou, G.L., 2009. Cloning, expression, characterization and phylogenetic analysis of arginine kinase from greasyback shrimp (*Metapenaeus ensis*). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 153 (3), 268–274.
- Wang, S., Delgado, J.C., Ravkov, E., Eckels, D.D., Georgelas, A., Pavlov, I.Y., Cusick, M., Sebastian, K., Gleich, G.J., Wagner, L.A., 2012. Penaeus monodon tropomyosin induces CD4 T-cell proliferation in shrimp-allergic patients. *Hum. Immunol.* 73 (4), 426–431.
- Wang, X., Li, L., Xu, F., Zhang, G., 2011c. Tropomyosin is a nice marker gene for phylogenetic analysis of molluscs. *Mol. Biol. Rep.* 38 (7), 4589–4593.
- Weber, P., Steinhart, H., Paschke, A., 2009. Competitive indirect ELISA for the determination of parvalbumins from various fish species in food grade fish gelatins and isinglass with PARV-19 anti-parvalbumin antibodies. *J. Agr. Food Chem.* 57 (23), 11328–11334.
- Wen, Y., Zhan, S., Huang, H., Zhong, M., Chen, J., You, C., Wang, F., Zhang, Y., 2016. Identification and characterization of an 18.4kDa antimicrobial truncation from shrimp *Litopenaeus vannamei* hemocyanin upon *Vibrio parahaemolyticus* infection. *Fish Shellfish Immunol.* 56, 450–458.
- Whitby, F.G., Phillips, G.N., 2000. Crystal structure of tropomyosin at 7 Å resolution. *Proteins-Struct. Funct. Genet.* 38 (1), 49–59.
- White, A.J., Northcutt, M.J., Rohrbach, S.E., Carpenter, R.O., Niehaus-Sauter, M.M., Gao, Y., Wheatly, M.G., Gillen, C.M., 2011. Characterization of sarcoplasmic calcium binding protein (SCP) variants from freshwater crayfish *Procambarus clarkii*. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 160 (1), 8–14.
- Wierenga, R.K., Kapetanios, E.G., Venkatesan, R., 2010. Triosephosphate isomerase: a highly evolved biocatalyst. *Cell Mol. Life Sci.* 67 (23), 3961–3982.
- Wnuk, W., Cox, J.A., Stein, E.A., 1981. Structural changes induced by calcium and magnesium in a high affinity calcium-binding protein from crayfish sarcoplasm. *J. Biol. Chem.* 256 (22), 11538–11544.
- Wnuk, W., Jauregui-Adell, J., 1983. Polymorphism in high-affinity calcium-binding proteins from crustacean sarcoplasm. *Eur. J. Biochem.* 131 (1), 177–182.
- Wu, T.C., Tsai, T.C., Huang, C.F., Chang, F.Y., Lin, C.C., Huang, I.F., Chu, C.H., Lau, B.H., Wu, L., Peng, H.J., Tang, R.B., 2012. Prevalence of food allergy in Taiwan: a questionnaire-based survey. *Intern. Med. J.* 42 (12), 1310–1315.
- Yadzir, Z.H., Misnan, R., Abdullah, N., Bakhtiar, F., Arip, M., Murad, S., 2012. Identification of the major allergen of *Macrobrachium rosenbergii* (giant freshwater prawn). *Asian Pac. J. Trop. Biomed.* 2 (1), 50–54.
- Yang, A.C., Arruda, L.K., Santos, A.B., Barbosa, M.C., Chapman, M.D., Galvao, C.E., Kalil, J., Morato-Castro, F.F., 2010. Measurement of IgE antibodies to shrimp tropomyosin is superior to skin prick testing with commercial extract and measurement of IgE to shrimp for predicting clinically relevant allergic reactions after shrimp ingestion. *J. Allergy Clin. Immunol.* 125 (4), 872–878.
- Yang, Y., Cao, M.-J., Alcocer, M., Liu, Q.-M., Fei, D.-X., Mao, H.-Y., Liu, G.-M., 2015. Mapping and characterization of antigenic epitopes of arginine kinase of *Scylla paramamosain*. *Mol. Immunol.* 65 (2), 310–320.
- Yang, Y., Chen, Z.W., Hurlburt, B.K., Li, G.L., Zhang, Y.X., Fei, D.X., Shen, H.W., Cao, M.J., Liu, G.M., 2017. Identification of triosephosphate isomerase as a novel allergen in *Octopus fangsiao*. *Mol. Immunol.* 85, 35–46.
- Yao, C.L., Ji, P.F., Kong, P., Wang, Z.Y., Xiang, J.H., 2009. Arginine kinase from *Litopenaeus vannamei*: cloning, expression and catalytic properties. *Fish Shellfish Immunol.* 26 (3), 553–558.
- Yoshida, S., Ichimura, A., Shiomi, K., 2008. Elucidation of a major IgE epitope of Pacific mackerel parvalbumin. *Food Chem.* 111 (4), 857–861.
- Yousef, M.S., Clark, S.A., Pruett, P.K., Somasundaram, T., Ellington, W.R., Chapman, M.S., 2003. Induced fit in guanidino kinases—comparison of substrate-free and transition state analog structures of arginine kinase. *Protein Sci.* 12 (1), 103–111.
- Yousef, M.S., Fabiola, F., Gattis, J.L., Somasundaram, T., Chapman, M.S., 2002. Refinement of the arginine kinase transition-state analogue complex at 1.2 Å resolution: mechanistic insights. *Acta Cryst. D58*, 2009–2017.
- Yu, C.J., Lin, Y.F., Chiang, B.L., Chow, L.P., 2003. Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *J. Immunol.* 170 (1), 445–453.
- Zhang, Y., Matsuo, H., Morita, E., 2006. Cross-reactivity among shrimp, crab and scallops in a patient with a seafood allergy. *J. Dermatol.* 33 (3), 174–177.
- Zhang, Y., Zhu, L., Li, S., Zhang, J., She, T., Yan, J., Bian, Y., Li, H., 2016. Identification of the major allergenic epitopes of *Eriocheir sinensis* roe hemocyanin: a novel tool for food allergy diagnoses. *Mol. Immunol.* 74, 125–132.
- Zhang, Y.X., Chen, H.L., Maleki, S.J., Cao, M.J., Zhang, L.J., Su, W.J., Liu, G.M., 2015. Purification, characterization, and analysis of the allergenic properties of myosin light chain in *procambarus clarkii*. *J. Agric. Food Chem.* 63 (27), 6271–6282.

- Zhang, Z., Wang, F., Chen, C., Zheng, Z., Aweya, J.J., Zhang, Y., 2017. Glycosylation of hemocyanin in *Litopenaeus vannamei* is an antibacterial response feature. *Immunol. Lett.* 192, 42–47.
- Zhou, G., Somasundaram, T., Blanc, E., Parthasarathy, G., Ellington, W.R., Chapman, M.S., 1998. Transition state structure of arginine kinase: implications for catalysis of bimolecular reactions. *Proc. Natl. Acad. Sci. U. S. A.* 95 (15), 8449–8454.
- Zhu, L., She, T., Zhang, Y., Li, S., Xu, Z., Yan, J., Li, H., 2016. Identification and characterization of ovary development-related protein EJO1 (Eri s 2) from the ovary of *Eriocheir sinensis* as a new food allergen. *Mol. Nutr. Food Res.* 60 (10), 2275–2287.
- Zuidmeer-Jongejan, L., Huber, H., Swoboda, I., Rigby, N., Versteeg, S.A., Jensen, B.M., Quaak, S., Akkerdaas, J.H., Blom, L., Asturias, J., Bindslev-Jensen, C., Bernardi, M.L., Clausen, M., Ferrara, R., Hauer, M., Heyse, J., Kopp, S., Kowalski, M.L., Lewandowska-Polak, A., Linhart, B., Maderegger, B., Maillere, B., Mari, A., Martinez, A., Mills, E.N., Neubauer, A., Nicoletti, C., Papadopoulos, N.G., Portoles, A., Ranta-Panula, V., Santos-Magadan, S., Schnoor, H.J., Sigurdardottir, S.T., Stahl-Skov, P., Stavroulakis, G., Stegellner, G., Vazquez-Cortes, S., Witten, M., Stolz, F., Poulsen, L.K., Fernandez-Rivas, M., Valenta, R., van Ree, R., 2015. Development of a hypoallergenic recombinant parvalbumin for first-in-man subcutaneous immunotherapy of fish allergy. *Int. Arch. Allergy Immunol.* 166 (1), 41–51.