

## Allergy to Black Bass Fin and Carp

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Fish allergy is a pathological, IgE-mediated immune response to specific fish proteins. Sensitization is mainly via exposure to the allergen the gastrointestinal tract, as well as via exposure to fish aeroallergens in the respiratory tract or through skin contact [1,2]. Most fish-allergic patients show marked clinically relevant cross-reactivity, while a minority of patients experience selective allergy to specific fish species, with good tolerance to other fish families [3].

Black bass (*Micropterus salmoides*) is originally from North America and was introduced in Spain in 1955 for recreational fishing. Like carp (Cyprinidae), it is predominant in freshwater fishing.

We present a case of contact allergy due to 2 different proteins of black bass and carp.

A 24-year-old recreational angler with a history of rhinoconjunctivitis and bronchial asthma due to sensitization to house dust mites and olive pollen experienced conjunctival erythema, nasal block-age, and dyspnea as a result of contact with cyprinids. Therefore, when he went fishing, he premedicated with oral antihistamines and salbutamol and used gloves. He was referred to our unit after an episode of hand edema and upper ipsilateral limb edema accompanied by generalized urticaria resulting from accidental puncture with the dorsal fin of a black bass. He presented no additional symptoms. The episode resolved spontaneously in a few minutes. He subsequently handled black bass without incident and tolerated fish intake.

The patient underwent skin prick tests (SPTs) for the most common aeroallergens in our area (mites, pollens, molds, latex, *Anisakis simplex*, and dander [cat, dog, and horse]) and also for hake and cod. His results were positive to *Dermatophagoides pteronyssinus*, *Glycyphagus domesticus*, and *Olea europaea* pollen.

We performed prick-by-prick tests with carp and black bass. The results were positive, with wheals measuring 11 mm and 7 mm, respectively.

Proteins from different parts of raw black bass and carp (fin, head, body, and skin) were extracted with phosphate-buffered saline (PBS) and then homogenized using a mortar and pestle at  $5 \pm 3^\circ\text{C}$ . The mix remained homogenized until the greatest possible content was released. The homogenate was

shaken magnetically before being sieved to remove nonsoluble material. The extract was then centrifuged, filtered, dialyzed against deionized water, and lyophilized.

Proteins were separated by SDS-PAGE in 15% polyacrylamide gels, which were loaded at 10  $\mu\text{g}/\text{well}$  according to the method described by Laemmli [4]. Subsequently, the patient's diluted serum (1:5) was incubated with the proteins (1  $\mu\text{g}/\text{well}$ ), which were transferred onto a polyvinylidene fluoride (PVDF) membrane as previously described [5]. After blocking with 0.5% PBS Tween-20 buffer, the membrane was incubated overnight with the patient's serum. A PVDF membrane containing the same extract was incubated with 0.5% PBS Tween-20 buffer and with serum from a nonatopic individual as a negative control. The PVDF membranes were incubated with mouse antihuman IgE Fc-HRP (Southern Biotech). Reactive bands were detected using enhanced chemiluminescence following the manufacturer's instructions (Western Lightning Plus-ECL, Perkin Elmer).

The Western blot analysis revealed IgE-binding bands for several proteins weighing between 45 and 25 kDa in the black bass fin, as well as a 25-kDa protein in all of the carp parts studied. While the molecular weight was similar to that of the black bass protein, the intensities varied, probably owing to differences in content (Figure).

Multiple IgE-binding bands have been detected in fish extracts, but parvalbumins are considered to be the most clinically relevant allergen [3,6,7]. Parvalbumin is an acidic calcium-binding protein of 113 amino acids that is very common in fish muscle. It has a low molecular weight of approximately 10-12 kDa [8,9]. It is highly stable, resistant to proteolysis, and can be transported in aerosols and cooking vapors [2]. In addition to the parvalbumins, several other fish proteins (eg, enolases, aldolases, and fish gelatin) have been identified as major fish allergens.

In the present case, we identified 2 previously undescribed allergenic proteins in 2 fish species. In the case of carp, we found an apolipoprotein B of 27.5 kDa with sequence coverage of 14% by peptide mass fingerprinting in all the parts studied. Sensitization seemed to be through contact with the animal and caused respiratory symptoms (rhinoconjunctival and asthma) and skin symptoms (urticaria in the contact area). In the case of black bass, however, the protein identified was a  $\beta$ -actin with a molecular mass of 42 kDa and sequence coverage of 38%, again using peptide mass fingerprinting. This protein

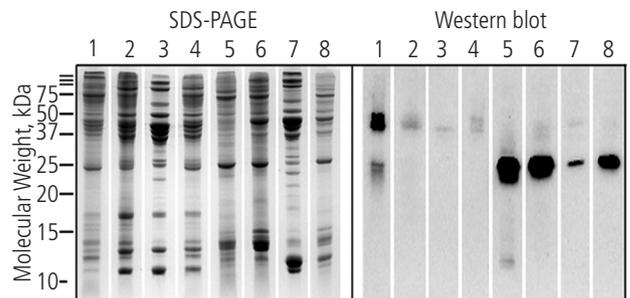


Figure. SDS-PAGE and Western blot: Lane 1, black bass fin; Lane 2, black bass head; Lane 3, black bass body; Lane 4, black bass skin; Lane 5, carp fin; Lane 6, carp head; Lane 7, carp body; Lane 8, skin.

was present exclusively in the fin. Furthermore, sensitization was by contact, and the only manifestations were cutaneous.

Cutaneous symptoms have previously been reported after contact with raw fish. Shimojo et al [1] provide data on a patient who presented with itching and urticaria on the forearms after contact with raw conger. An immunoblot study revealed IgE against  $\alpha$ -actin-3 of approximately 110 kDa. This protein has been described as a cross-reactive protein in house dust mites and in shrimp [10]. In the present case, contact with carp induced not only cutaneous symptoms, but also a systemic reaction. To our knowledge, this is the first report of anaphylaxis due to cutaneous contact with cyprinids, although there are reports of anaphylactic reactions to seafood [3]. The allergenic proteins have a lower molecular weight than in the previously mentioned case. Furthermore, the patient reacted to 2 different fish species and allergens.

In conclusion, we identified 2 proteins with IgE binding capacity that had not been previously described in different species of fish. Both bands could correspond to a new fish allergen family, although further characterization is necessary.

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#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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