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Allergenicity and Stability of 6 New Korean Bony Fish Extracts

Ji Eun Yuk,[1] **Jongsun Lee**,[1] **Kyoung Yong Jeong**,[1] **Kyung Hee Park**,[1] **Jung Dong Kim**,[2] **Ji-Tae Kim**,[2] **Jae-Hyun Lee**,[1] **Jung-Won Park**[1,2*]

[1]Division of Allergy and Immunology, Department of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea
[2]Prolagen, Seoul, Korea

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Correspondence to
Jung-Won Park, MD, PhD
Division of Allergy and Immunology, Department of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.
Tel: +82-2-2228-1961
Fax: +82-2-393-6884
E-mail: parkjw@yuhs.ac

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ORCID iDs
Ji Eun Yuk
https://orcid.org/0000-0003-2517-2653
Jongsun Lee
https://orcid.org/0000-0003-1526-2241
Kyoung Yong Jeong
https://orcid.org/0000-0001-9887-1426
Kyung Hee Park
https://orcid.org/0000-0003-3605-5364
Jung Dong Kim
https://orcid.org/0000-0002-6348-9267
Ji-Tae Kim
https://orcid.org/0000-0002-8690-358X

ABSTRACT

Purpose: Diagnostic tests for allergen sensitization should reflect real exposure. We made 6 new bony fish extracts, which are consumed popularly in Korea, and evaluated their allergenicity and stability.
Methods: We manufactured fish extracts from codfish, mackerel, common eel, flounder, cutlass, and catfish. Protein and parvalbumin (PV) were evaluated by Bradford assay, 2-site enzyme-linked immunosorbent assay, sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), and anti-PV immunoblotting. The immunoglobulin E (IgE) reactivities of the extracts were evaluated with ImmunoCAP and IgE immunoblotting using sera from 24 Korean fish allergy patients, 5 asymptomatic sensitizers, and 11 non-atopic subjects. Stability of the extracts stored in 4 different buffers were evaluated for up to a year.
Results: The protein concentrations of commercial SPT fish extracts varied with up to a 7.5-fold difference. SDS-PAGE showed marked differences in the PV concentrations of commercial SPT reagents. Specific IgE measurements for the following investigatory fish extracts—iCodfish, iMackerel, and iEel—were concordant with that of their corresponding Phadia ImmunoCAP measurements. ImmunoCAP results showed marked IgE cross-reactivity among the fish species, and the overall sensitivity of ImmunoCAP with the investigatory fish extracts for identification of culprit fish species was 85.7%. The protein and PV concentrations in the investigatory extracts were highly stable in saline with 0.3% phenol–50% glycerol at 4°C for up to a year.
Conclusions: The commercial SPT fish extracts exhibited considerable variation in terms of allergenicity, which may impact on diagnostic accuracy. Our new fish extracts have sufficient allergenicity and stability and may be adequate to various clinical applications.

Keywords: Allergenicity; bony fish; extracts; food allergy; fish allergy; fish parvalbumin

INTRODUCTION

Fish represent a major source of animal protein for human nutrition. As the consumption of fish increases, the prevalence of fish allergy increases. Notably, the prevalence of fish allergy varies among countries, as well. Meta-analysis showed that the prevalence of sensitized and symptomatic fish allergy was less than 0.5%.[1] In Korea, fish are also well recognized as an

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Jae-Hyun Lee
https://orcid.org/0000-0002-0760-0071
Jung-Won Park
https://orcid.org/0000-0003-0249-8749

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important culprit food allergen that affects both children and adults,[2-4] and the prevalence of confirmed or possible fish allergy among children and adolescents was estimated to be 0.09%.[4] Fish are classified as bony or cartilaginous; however, most edible fish are classified as bony. Previous studies identified parvalbumin (PV),[5] tropomyosin,[6] aldolase A,[7] β-enolase,[7] vitellogenin, and collagen as fish allergens.[8] Among them, PV is recognized as a major fish allergen. Fish PVs are 10–15 kDa proteins that are subdivided into α- and β-isoforms, and the β-isoforms are predominantly found in the muscle tissue of bony fish.[8] PVs are heat-stable, calcium-binding muscle proteins that are resistant to protease and chemical denaturation.[5] Clinical history and skin prick test (SPT) results of fish allergy patients showed that most fish allergies are induced by bony fish species and that there is a significant cross-sensitivity among bony fish species.[9,10] Thus, some physicians recommend that patients with fish allergies avoid all fish.[11] However, strict fish avoidance has its disadvantages and may lead to nutritional imbalances because fish offer significant nutritional benefits that prevent various diseases.[12,13] Fish allergies to cartilaginous fish species are rarely reported, and there is little cross-reactivity between bony and cartilaginous fishes.[8] This may be because cartilaginous fish express low levels of allergenic β-PVs and high levels of non-allergenic α-PVs. Furthermore, many patients with fish allergies are able to tolerate some fish species, perhaps due to species-specific fish allergen sensitization.[7,14,15] Clinical cross-reactivity to other fish species has been reported in 50% of fish-allergic patients.[9,14,16] Many double-blind placebo-controlled food challenge tests reported different clinical sensitivities to some fish species, even though SPT or specific immunoglobulin E (sIgE) tests showed similar positive sensitivities to various fish species.[16-18] A diagnosis of allergies to commonly consumed fish species, which reflects real exposure, is essential to provide more precise avoidance recommendations. In Korea, most fish species are supplied by wild-catching marine fish or aquaculture. Among wild-catching fish species, pollack which is quite similar to codfish, mackerel, and cutlass are popularly consumed. Flounder and eel, which provided by fish aquaculture, are also very popular.[19,20] In Korea, diagnostic tests rely on imported allergen extracts; thus, the diagnostic panels for fish allergy may not include the commonly consumed fish species in Korea. This discrepancy between the diagnostic panel and real exposure likely affects the accuracy of fish allergy diagnoses.

In this study, we made 6 new extracts of bony fishes, such as codfish, mackerel, eel, flounder, cutlass, and catfish, which are commonly consumed in Korea. Then, we evaluated their allergenicity in comparison with commercially available SPT reagents of bony fish species and tested the stability of our fish extracts for clinical application.

MATERIALS AND METHODS

Sera from allergy patients

Sera from 29 atopic patients and 11 non-atopic control subjects were used to measure sIgE levels for 6 different fish species extracts using the ImmunoCAP platform. This study was approved by Institutional Review Boards (approval No. 4-2017-0588). Among the 29 patients, 24 exhibited immediate-type allergic symptoms, such as onset or aggravation of atopic dermatitis (13 patients), angioedema/urticaria (15 patients), anaphylaxis (2 patients), and eosinophilic esophagitis and urticaria (1 patient) after ingestion of fish species and had positive sIgEs to fish species. Seven of 24 patients had 2 fish-allergic diseases. The other 5 patients also had sIgEs to fish species, but did not complain of specific allergic symptoms after ingestion of fish.

Allergen extracts

Six fish species—iCodfish (*Gadus macrocephalus*, Pacific codfish), iMackerel (*Scomber japonicus*, Chub mackerel), iEel (*Anguilla japonica*), iFlounder (*Paralichthys olivaceus*), iCutlass (*Trichiurus lepturus*, largehead hairtail), and iCatfish (*Silurus asotus*, far Eastern catfish)—were purchased from a local Korean market. All the fish except catfish and eel were captured from the sea near the Korean peninsula. The catfish was captured from a freshwater river in Korea and eel was aquacultured in fresh water.

To prepare allergen extracts, 200–350 g of muscle were excised using forceps, scalpels, and scissors, which were boiled for 10 minutes in distilled water (DW). The boiled muscle tissue was homogenized in phosphate buffered saline (PBS, 1:4 w/v) in a Waring blender (Kenwood, Birmingham, UK) and then lyophilized. After defatting the tissue with ethyl ether (1:4 w/v), allergens were extracted in PBS (1:4 w/v) at 4°C for 24 hours, and centrifuged at 12,000 rpm at 4°C for 15 minutes. The supernatants were dialyzed extensively against DW (cutoff 3.5 kDa; Spectrum, New Brunswick, NJ, USA). After dialysis, the extracts were filter-sterilized using 0.22-μm Millipore syringe filters (Merk, Darmstadt, Germany), lyophilized, and stored at –76°C till use.

For comparison, SPT reagents for codfish, mackerel, and common eel were obtained from Allergopharma GmbH & Co. KG (Reinbek, Germany), Lofarma (Milan, Italy), and Hollister-Stier LLC (Spokane, WA, USA).

Measurement of protein and PV content

Protein content and PV concentration were measured by Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA) using bovine serum albumin as a standard and the Fish PV enzyme-linked immunosorbent assay (ELISA) kit (Demeditec Diagnostic GmbH, Kiel, Germany), respectively. The detection limit of the ELISA kit was 1.2 ng/mL.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Allergen extracts (20 μL/well of the commercial SPT extracts and the new fish extracts shown in **Table 1**) were separated on an 18% SDS-PAGE under reducing conditions. The gels were stained with Coomassie brilliant blue or transferred onto polyvinylidene difluoride membranes (GE Waters & Process Technologies, Trevose, PA, USA) for immunoblotting. For the detection of IgE-reactive components, membranes were incubated overnight with pooled sera from the allergic patients (1:4 dilution) at room temperature, after blocking with 3% skim milk. Then, membranes were incubated with alkaline phosphatase-conjugated goat

Table 1. Protein and PV concentrations of the 6 new investigatory and commercial skin prick test fish reagents measured by Bradford assay and 2-site enzyme-linked immunosorbent assay

Allergen	Protein concentration (μg/mL)				PV concentration (ng/mL)			
	Allergopharma	Lofarma	Hollister-Stier	New extracts	Allergopharma	Lofarma	Hollister-Stier	New extracts
					Converted value* (raw data)			
Codfish	294.7	445.1	859.6	1,081.5	91.9 (91.9)	345.1 (345.1)	403.8 (403.8)	155.8 (155.8)
Mackerel	NA	518.8	1,808.3	980.8	NA	3,912.0 (78.2)	5,305.1 (106.1)	5,745.8 (114.9)
Common eel	248.2	241.5	NA	1,008.3	1,960.7 (67.6)	3,146.7 (108.5)	NA	4,867.5 (167.8)
Flounder	300.9	NA	NA	1,017.5	484.2 (68.2)	NA	NA	724.8 (102.1)
Cutlass	NA	NA	NA	878.9	NA	NA	NA	- (179.4)
Catfish	NA	NA	NA	1,055.0	NA	NA	NA	166.0 (97.6)

PV, parvalbumin; NA, not available.
*Conversion factor for each fish species is 1.0 for codfish, 50.0 for mackerel, 29.0 for eel, 7.1 for flounder, and 1.7 for catfish. It is not available for cutlass.

anti-human IgE (1:1,000 dilution, ε-chain specific; Sigma-Aldrich, St. Louis, MO, USA) and colorimetric detection was performed with nitroblue tetrazolium (NBT) and 3-bromo-4-chloro-5-indolyl-phosphate (BCIP; Promega, Madison, WI, USA). Membranes were washed with Tris-buffered saline containing 0.05% Tween 20 (pH 8.0) between individual steps.

For the detection of PV, membranes were incubated with an anti-frog PV monoclonal antibody (mAb) PARV-19 (1:1,000 dilution; Sigma-Aldrich) for 1 hour.[21] Immunoreactivity was detected with alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma-Aldrich) and NBT/BCIP.

Measurement of fish-specific IgEs by ImmunoCAP analysis

Fish sIgEs were measured using the ImmunoCAP assay platform (Phadia, Uppsala, Sweden) to compare allergenicity between the extracts. For ImmunoCAP analysis, biotinylation of the fish extracts was performed with EZ-link® Sulfo-NHS-LC-Biotin (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, extracts (2 mg) were incubated with NHS-LC-biotin on ice for 4 hours, and then dialyzed extensively against PBS to remove unreacted NHS-LC-biotin. Biotinylated fish extracts were loaded onto streptavidin ImmunoCAPs. IgE antibody binding to the extracts was measured using the Phadia UniCAP 100 system according to the manufacturer's instructions. A sIgE value of ≥ 0.35 kU/L was considered positive.

Stability of fish extracts

Lyophilized investigatory fish extracts each were dissolved in normal saline, saline with 50% glycerol, saline with 0.3% phenol, and saline with 0.3% phenol–50% glycerol. Reconstituted extracts were aliquoted and stored at 4°C or room temperature (18°C–26°C). Samples were removed after 1, 2, 4, 9, 13, 26, and 52 weeks and stored at −76°C for stability analysis.

Statistical analysis

Concordance rates for positive and negative ImmunoCAP tests were evaluated by Pearson χ^2 analysis with Fisher's exact test. Correlations between ImmunoCAP results for Thermo Fisher and the investigatory extracts were evaluated by Spearman's rank correlation test. P values < 0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA).

RESULTS

Protein analysis of 6 new bony fish extracts

We measured the protein concentrations of commercially available SPT reagents and of the 6 new fish extracts by Bradford assay. The protein concentrations of commercial SPT reagents ranged from 241.5 to 1,808.3 μg/mL, which differed by up to 7.5 fold in their protein concentration. However, the protein concentrations of the new SPT extracts ranged from 878.9 to 1,081.5 μg/mL We measured PV concentrations using 2-site ELISA kit. The mAbs used in this kit have different affinity for PVs, so the manufacturer recommends you to apply fish species-specific conversion factors to estimate the concentrations of PVs. Estimated PV concentrations of the new extracts ranged from 155.8–5,745.8 ng/mL (**Table 1**). These values were higher than or equal to those of commercial SPT reagents. PV of iCutlass could not be estimated due to the lack of conversion factor. SDS-PAGE showed marked differences in β-PV (10–15 kDa) concentrations among the commercial SPT fish extracts, and the PV concentrations were higher in the new extracts (**Fig. 1A**). Anti-PV immunoblotting with a mAb raised against frog muscle PV, which recognizes both α- and β-PVs, showed strong mAb

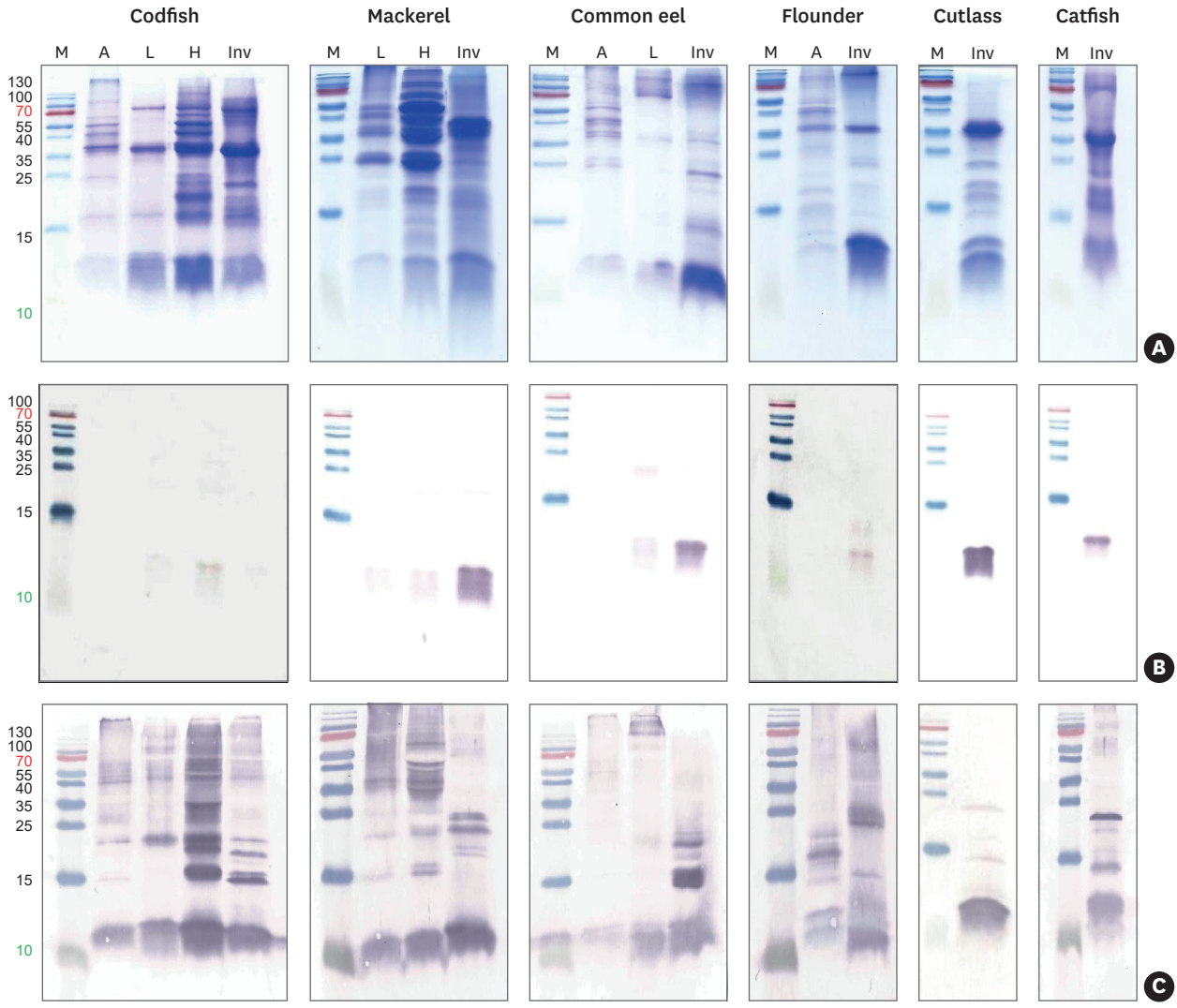

Fig. 1. SDS-PAGE (A), anti-parvalbumin IgG immunoblotting (B), and IgE immunoblotting with pooled sera from fish allergy patients (C) of fish extracts. Each lane of the SDS-PAGE gel was loaded with 20 μL of the commercial skin prick test reagents or the new extracts listed in **Table 1**.
M, marker; A, allergopharma; L, lofarma; H, Hollister-Stier; Inv, investigatory; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; Ig, immunoglobulin.

binding to PVs in the new bony fish extracts (**Fig. 1B**). IgE immunoblotting with pooled sera from fish allergy patients showed a similarly strong IgE binding to β-PVs in the 6 new fish extracts (**Fig. 1C**).

ImmunoCAP measurements of sIgEs to Phadia and the new bony fish allergens

We used the ImmunoCAP system to measure sIgE levels of the 6 new bony fish extracts using the sera of 24 fish-allergic patients, 5 asymptomatic sensitized patients and 11 negative subjects. The sIgE levels of the new extracts were < 0.1 kU/L in all the 11 negative serum samples.

ImmunoCAP results of 29 sera of atopic or asymptomatic sensitizer are shown in **Fig. 2A**. The numbers of negative results for each allergen were as follows: Phadia codfish (0), Phadia mackerel (3), Phadia eel (1), iCodfish (6), iMackerel (4), iEel (5), iFlounder (4), iCutlass (3), and iCatfish (1). Twenty-four allergic patients had complained of immediate allergic

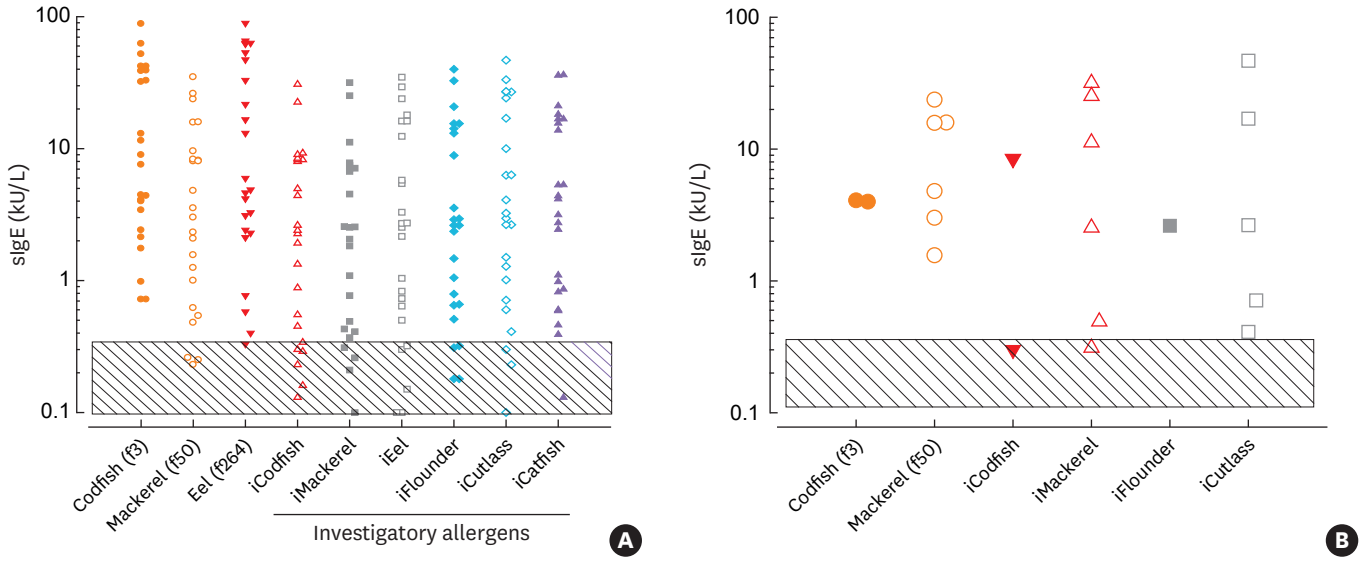

Fig. 2. ImmunoCAP measurements for 6 new fish extracts. (A) All ImmunoCAP measurements of 24 fish allergy patients and 5 asymptomatic sensitizers. (B) The ImmunoCAP measurements for 14 corresponding culprit allergens to the 11 patients' claim. Hatched box in the figures indicate negative range for ImmunoCAP measurement (< 0.35 kU/L).
sIgE, specific immunoglobulin E.

symptoms after ingestions of fish. Among them, only 11 could indicate their 14 suspected culprit fish species. The remaining 13 patients complained of fish allergy without detailed information on culprit fish species. We analyzed the ImmunoCAP results to the 14 suspected culprit fish species with 11 sera: codfish (2), mackerel (6), flounder (1), and cutlass (5). ImmunoCAP measurement with the new fish extracts showed 12 (85.7%) positive results to the corresponding suspected culprit allergens (**Fig. 2B**).

The χ^2 and Spearman's rank correlation analyses indicated that the sIgE levels of the new iCodfish, iMackerel, and iEel extracts strongly correlated with the those of the corresponding Phadia ImmunoCAP fish species: codfish (f3), mackerel (f50), and common eel (f264) (**Fig. 3**, **Table 2**). The correlations of sIgEs between the new fish extracts were strong with Spearman's rank correlation coefficient higher than 0.93 (**Table 2**).

Long-term stability of the new bony fish extracts

We evaluated the long-term stability of the 6 new fish extracts in 4 different buffers: normal saline, saline with 50% glycerol, saline with 0.3% phenol, and saline with 0.3% phenol–50% glycerol. The extracts, in various buffers, were stored at room temperature or 4°C for up to 1 year. In the saline with 50% glycerol, and saline with 0.3% phenol–50% glycerol buffers, protein concentrations were preserved for up to 1 year at room temperature (**Fig. 4A-F**). At 4°C, protein concentrations were preserved for up to 1 year in all 4 buffers (**Fig. 4G-L**). Extracts stored in saline with 0.3% phenol–50% glycerol, which is widely used in SPT reagents, showed attenuated SDS-PAGE patterns after 1 year at room temperature (**Fig. 5A-F**), but if stored at 4°C, it showed minimally changed SDS-PAGE patterns for up to 1 year (**Fig. 5G-L**). The SDS-PAGE features of the new fish allergen extracts stored in saline (**Supplementary Fig. 1A-L**), saline with 50% glycerol (**Supplementary Fig. S2A-L**), and saline with 0.3% phenol (**Supplementary Fig. S3A-L**) at room temperature or 4°C, respectively, for up to 1 year were also shown.

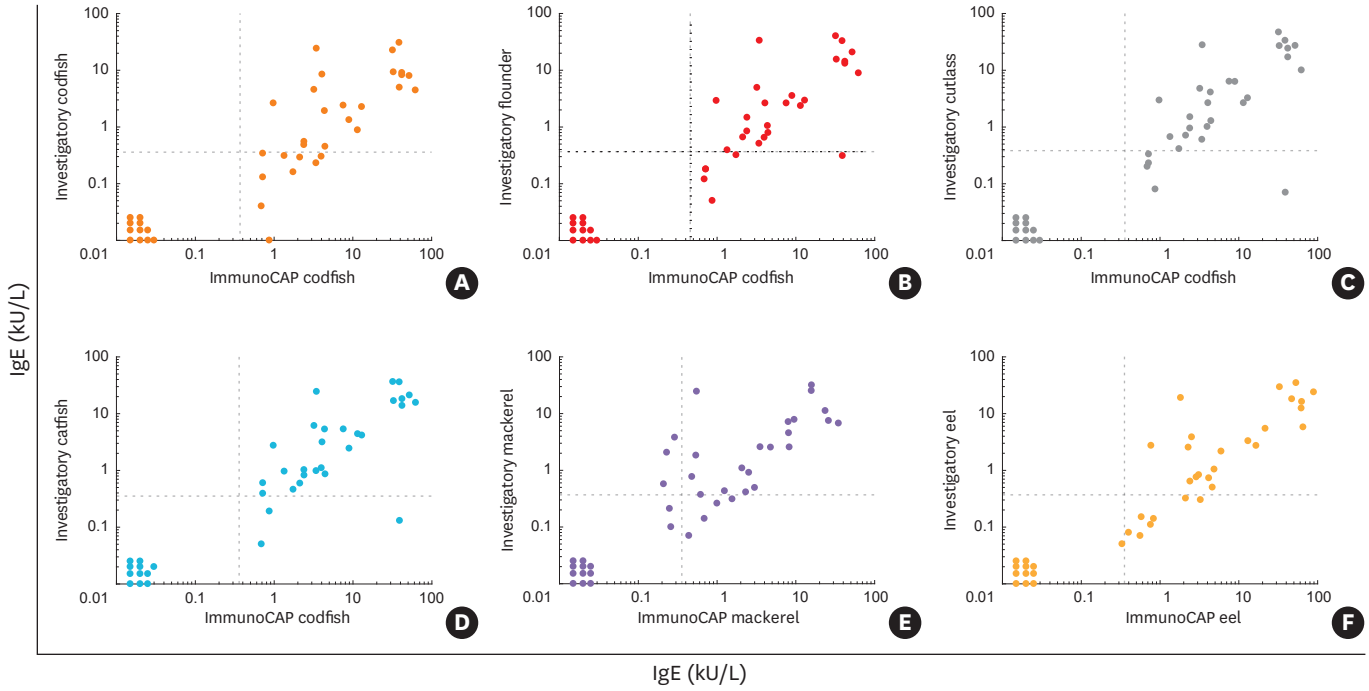

Fig. 3. sIgE levels against 3 Phadia ImmunoCAP fish species and the 6 new fish extracts in sera from 24 fish allergy patients, 5 asymptomatic sensitizers and 11 negative controls. All of the sIgE measurements were performed using the ImmunoCAP platform.
IgE, immunoglobulin E; sIgE, specific immunoglobulin E.

Table 2. The χ^2 and Spearman's rank correlation analyses of specific immunoglobulin E levels of Phadia ImmunoCAP fish species with those of the new fish extracts

	Concordance rate (%)	*P* value	Spearman's ρ	*P* value
Codfish/iCodfish	80.0	< 0.001	0.896	< 0.001
Codfish/iMackerel	85.0	< 0.001	0.904	< 0.001
Codfish/iEel	80.0	< 0.001	0.893	< 0.001
Codfish/iFlounder	85.0	< 0.001	0.893	< 0.001
Codfish/iCutlass	87.5	< 0.001	0.883	< 0.001
Codfish/iCatfish	92.5	< 0.001	0.889	< 0.001
iCodfish/iMackerel	90.0	< 0.001	0.958	< 0.001
iCodfish/iEel	92.5	< 0.001	0.938	< 0.001
iCodfish/iFlounder	85.0	< 0.001	0.951	< 0.001
iCodfish/iCutlass	82.5	< 0.001	0.944	< 0.001
iCodfish/iCatfish	77.5	< 0.001	0.938	< 0.001
Mackerel/iMackerel	82.5	< 0.001	0.883	< 0.001
iMackerel/iEel	95.0	< 0.001	0.984	< 0.001
iMackerel/iFlounder	95.0	< 0.001	0.987	< 0.001
iMackerel/iCutlass	92.5	< 0.001	0.977	< 0.001
iMackerel/iCatfish	87.5	< 0.001	0.981	< 0.001
Eel/iEel	82.5	< 0.001	0.921	< 0.001
iEel/iFlounder	95.0	< 0.001	0.995	< 0.001
iEel/iCutlass	92.5	< 0.001	0.984	< 0.001
iEel/iCatfish	87.5	< 0.001	0.972	< 0.001

i, investigatory.

Anti-PV mAb immunoblotting results were consistent with those of the SDS-PAGE. PV-specific bands were preserved up to a year when extracts were stored in saline with 0.3% phenol–50% glycerol at 4°C but the bands became progressively attenuated if the extracts were stored at room temperature (**Fig. 6A-L**). Anti-PV IgG immunoblotting of these extracts stored in normal saline (**Supplementary Fig. S4A-L**), normal saline with 50% glycerol

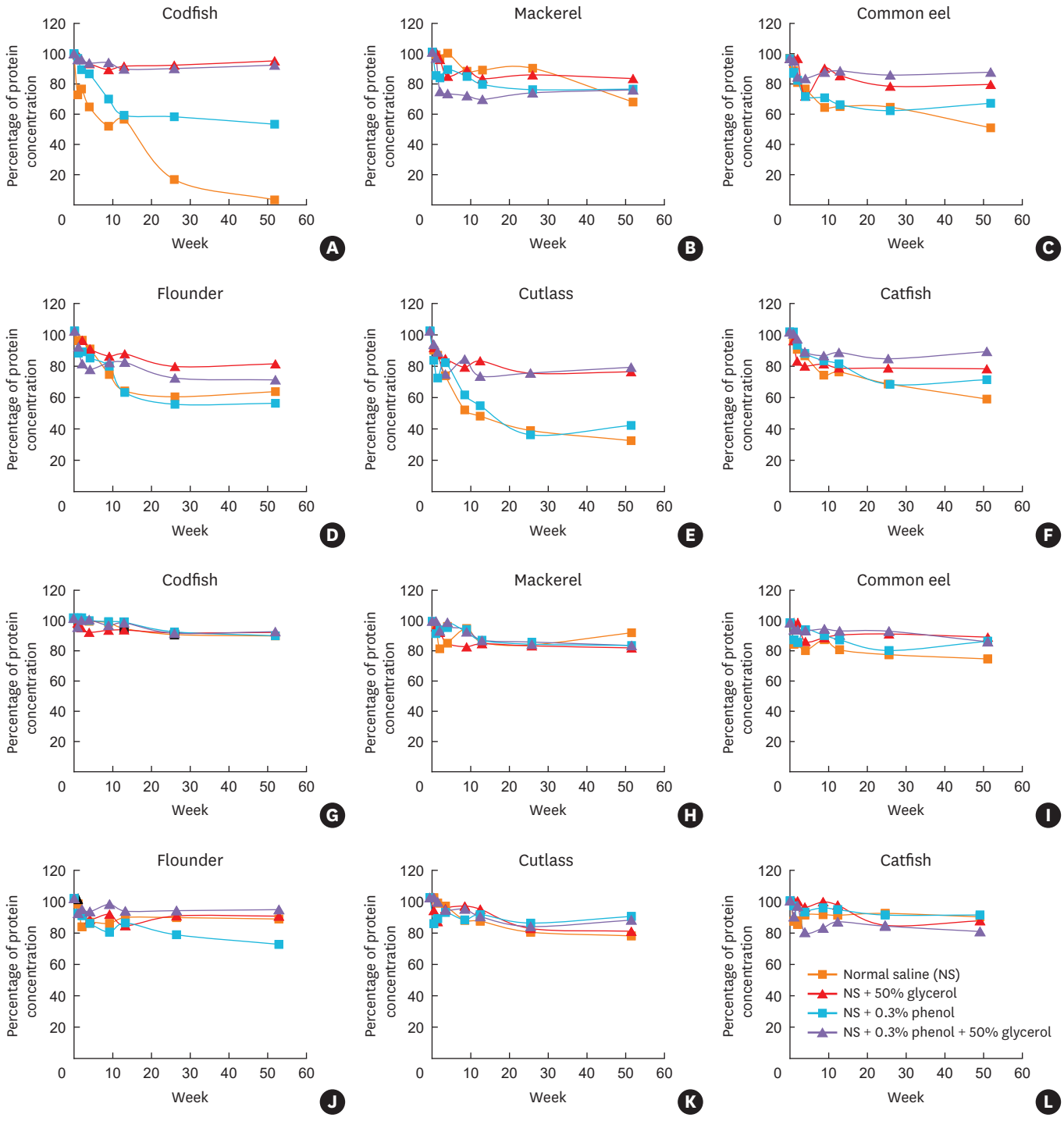

Fig. 4. Protein stability of the new fish extracts stored at room temperature (A-F) and 4°C (G-L) for up to a year. Extracts were stored in 4 different buffer compositions: normal saline, saline with 50% glycerol, saline with 0.3% phenol, and saline with 0.3% phenol–50% glycerol.

(**Supplementary Fig. S5A-L**), and normal saline with 0.3% phenol (**Supplementary Fig. 6A-L**) at room temperature or 4°C, respectively, for up to 1 year were also shown.

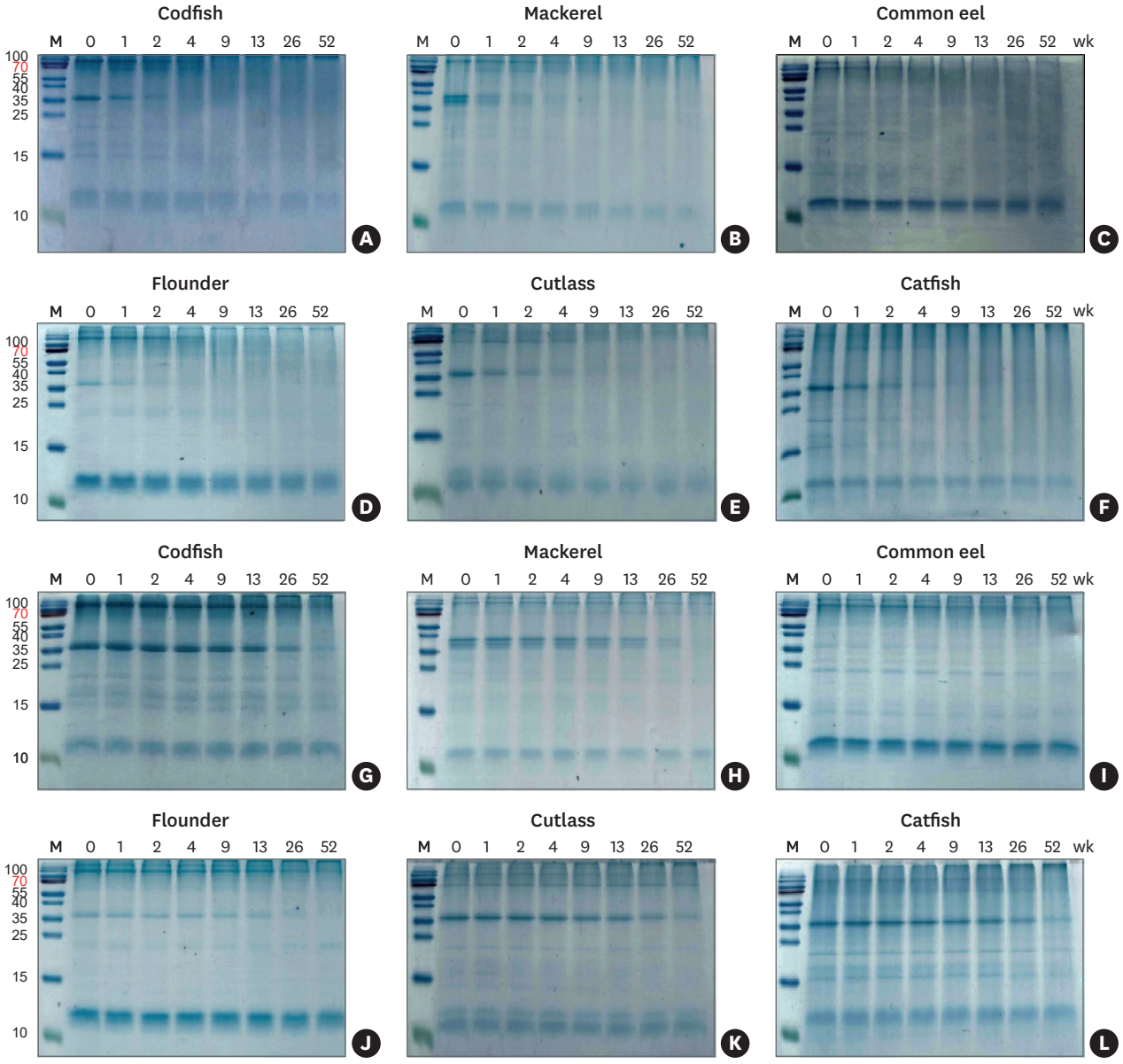

Fig. 5. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the new fish extracts stored in saline with 0.3% phenol–50% glycerol at room temperature (A-F) and 4°C (G-L) for up to a year.
M, marker.

Two-site ELISA measurements for PVs in the new fish extracts, stored in saline with 0.3% phenol–50% glycerol, were consistent with those of anti-PV mAb immunoblotting. The PV concentrations of the extracts were maintained for up to 1 year when stored at 4°C; however, it decreased progressively when stored at room temperature (**Fig. 7**).

DISCUSSION

In this study, it was demonstrated the allergenicity and stability of 6 new bony fish allergen extracts using the Bradford assay, SDS-PAGE, and immunoblotting with pooled sera from

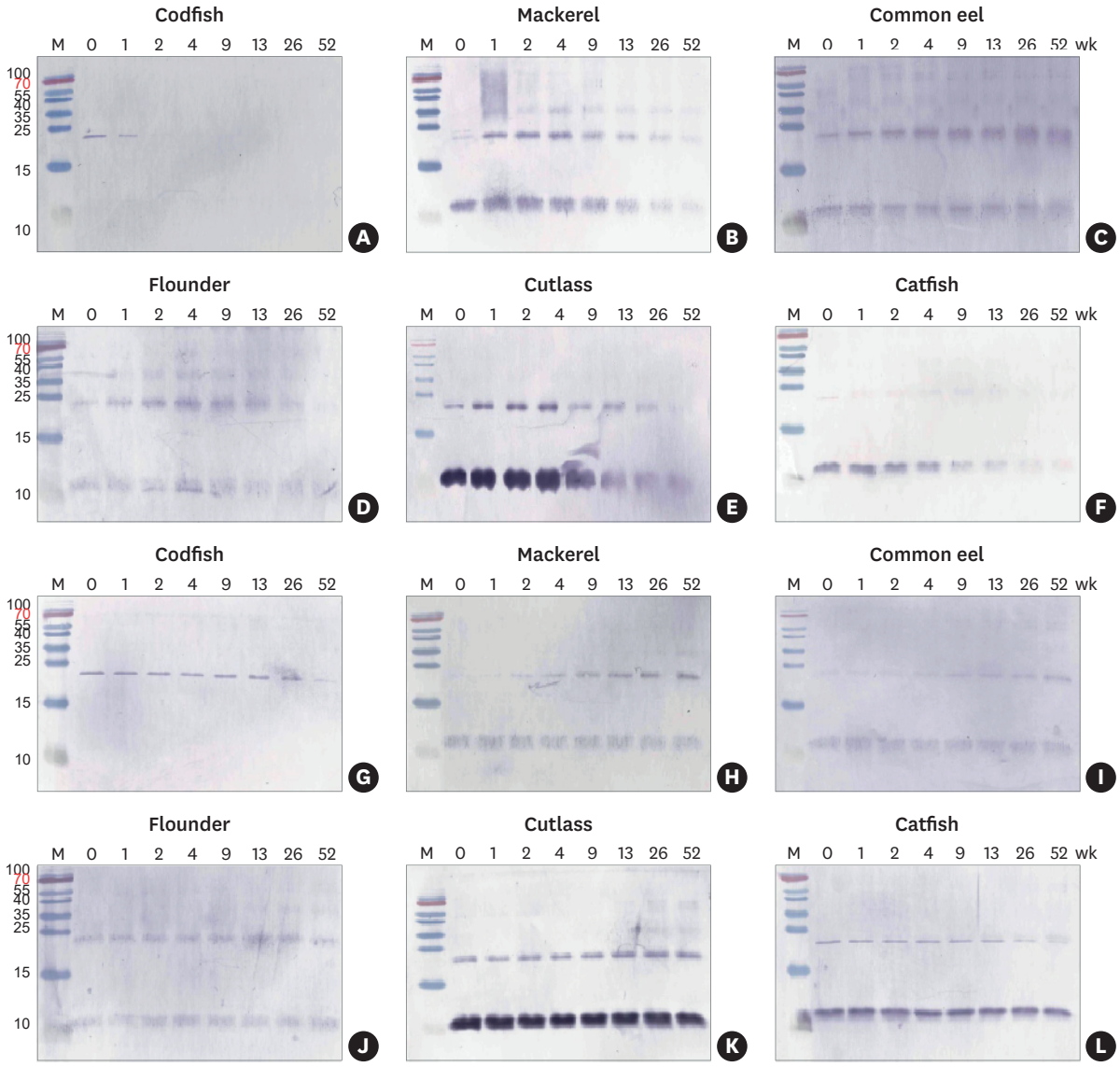

Fig. 6. Anti-parvalbumin immunoglobulin G immunoblotting of the new fish extracts stored in saline with 0.3% phenol–50% glycerol at room temperature (A-F) and 4°C (G-L) for up to a year.
M, marker.

Korean fish-allergic patients or an anti-PV mAb. Interestingly, it was found that the protein concentrations of commercial SPT fish extracts are quite variable with up to a 7.5-fold difference, but those of the new extracts were under control. The PV content in the muscle tissue of fish species is known to be considerably variable, and this feature may explain variations in allergic reactions to different fish species.[22] However, considerable variations in the PV content were also found among the same fish species. For example, previous studies showed low PV levels in commercial flounder extracts,[10,23] but our new iFlounder extract had a high PV concentration based on 2-site ELISA, SDS-PAGE, and immunoblotting data. Similarly, the PV content was lower in commercial common eel SPT extracts than in the iEel extract. The differences in PV level may be due to the lack of standardization in extract preparation or to differences in the species used. Thus, the standardization of SPT reagent preparation for fish allergen testing is urgently needed for reliable diagnosis.

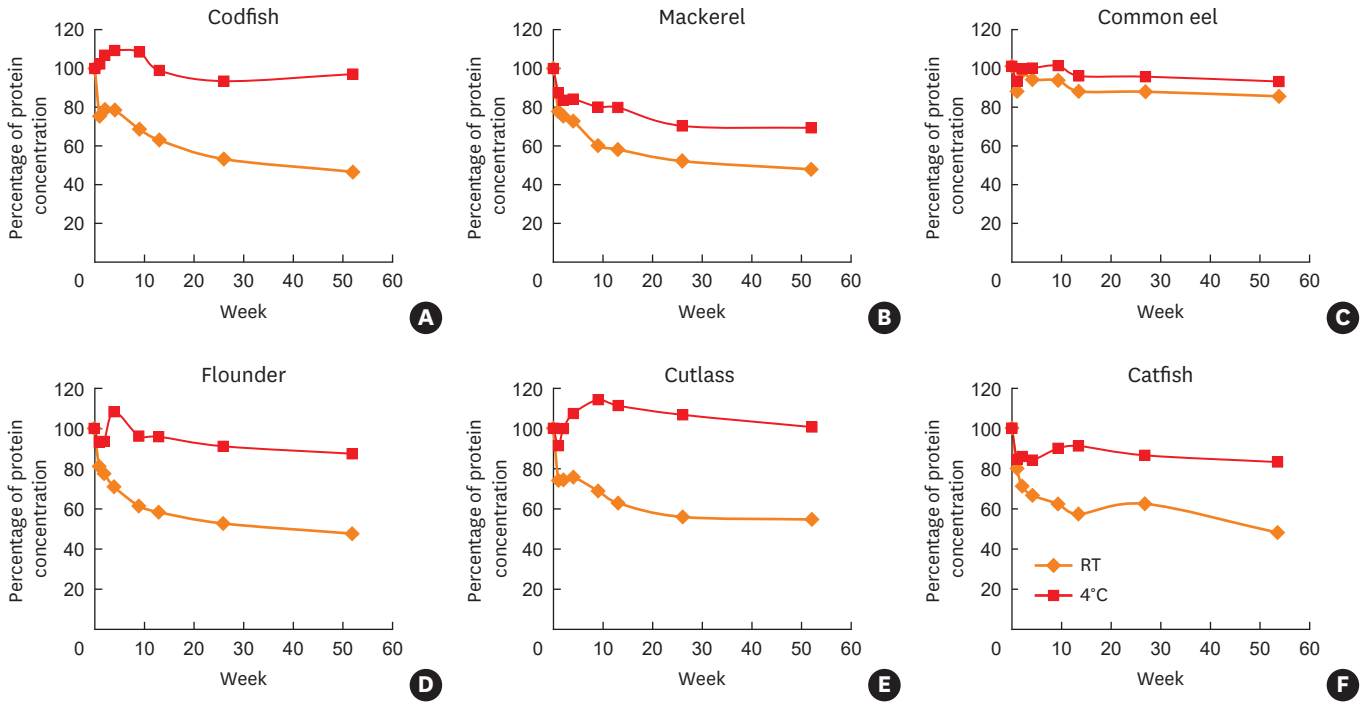

Fig. 7. Two-site enzyme-linked immunosorbent assay measurements of parvalbumins in the new fish extracts stored at room temperature and 4°C (A-F) for up to a year.

The allergenicity of fish may differ, depending on the species used, but commercial SPT companies do not provide information on the species used to prepare the extracts. The use of regional dominant fish species may enhance the diagnostic accuracy of SPT in the corresponding regions.

We made 6 bony fish allergen extracts because bony fish species are known to be the main cause of fish allergies worldwide.[10] Comparison analysis showed that the sIgE levels of the new extracts were concordant with those of the corresponding Phadia ImmunoCAP fish species, indicating that the new extracts are appropriate for clinical application. The stability of the new extracts in saline with 0.3% phenol–50% glycerol is important because this buffer composition is typically used for SPT reagents. Our new SPT extracts were found to be stable for up to 1 year when stored at 4°C, which may allow these new extracts to play multiple roles in real clinical practice.

PVs are regarded as major fish allergens and are the cause of extensive cross-reactivity to bony fish species, and many allergists have used codfish as the representative fish species for the diagnosis of fish allergy.[14] Our data also support strong cross-reactivity among the fish species. Surprisingly there was no significant difference in the positive rates of 6 different species with 29 atopic sera in this study. The positive rate for iCatfish, which lives in fresh water, was higher than that for the other sea water fish species. This result suggests the extensive cross-reactivity between sea and fresh water fish species, and that catfish or cutlass allergen extracts may be used as one of the representative fish allergens. Extensive homology of PVs between codfish (Gal c 1) and carp (Cyp c 1), a fresh water fish, has already been reported.[18,24]

In this study, it was confirmed the presence of PVs in fish extracts by SDS-PAGE and immunoblotting with an mAb against frog α- and β-PV. Frog α-PV shares IgE epitopes with codfish β-PV,[25] and the mAb used in this study also has affinity for mackerel β-PV. However, because the affinity for this mAb to the PVs of the 6 fish species extracts was quite variable, it may not be appropriate for quantifying PVs by direct comparison of the 2-site ELISA measurements. The manufacturer of the 2-site ELISA provides conversion factors for each fish species, reflecting the affinity of the mAbs. The estimated concentrations of PVs in the new extracts were higher than or equal to those in the commercial fish extracts.

Although PVs show strong cross-reactivity, species-specific fish sensitization has also been reported.[18,26] This species-specific sensitization may be due to sensitization to minor allergens, rather than pan-allergen β-PV or to sensitization to less cross-reactive PVs. The concentration of PVs in muscle tissue of flounder, tuna, and mackerel are lower compared to other fish species,[22,27] indicating that the former are a less allergenic fish species. In addition to PV, there are other fish allergens, such as collagen, tropomyosin, aldolase A, and β-enolase, that have limited cross-reactivities.[7] These allergens may trigger symptoms for fish allergy patients with mono-sensitization, who are tolerant to other fish species.[7] The identification of species-specific sensitization may require diagnostic tests with the extracts from a variety of commonly consumed regional fish species.[18] In a Japanese study, tuna, mackerel, and salmon were reported as highly allergenic fish species, although tuna and mackerel are known as less allergenic fish species in western countries.[22,27] This discrepancy may be attributed to the popular consumption of the former in Japan.[10] Contrary to Japan or western countries, grass carp, a fresh water fish, was the most common causative fish species in China.[18]

In Korea, pollack, codfish, mackerel, cutlass, eel, flounder, and catfish are the popular fish species to consume; diagnostic tests for these fish species may be important to recommend patients to avoid which culprit fish species. However, the results of sIgE measurement showed little difference in positive rates to the fish species in this study. Previous studies showed that SPT or sIgE measurement is not sufficient for the identification of culprit fish species due to the presence of strong cross-reactivity between PVs. Bernhisel-Broadbent *et al.*[17] reported that there was no difference in 50% ELISA inhibitory concentration between symptomatic and asymptomatic fish species. Fish-allergic patients usually have SPT positive response to various fish species, but about 50% of the patients were tolerable to at least more than 1 fish species in double-blind placebo-controlled food challenge tests, even they were positive to the skin prick test.[9,16] Our study also supports the insufficiency of sIgE measurement for the identification of culprit fish species and other diagnostic approaches, such as oral provocation test or basophil activation test, are required for tailored managements in fish-allergic patients.[16,18,28]

Some limitations exist in this study. A significant weakness is that we did not measure the levels of the minor allergens, which may be critical for mono-sensitized fish-allergic patients. For those kinds of studies, however, obtaining the sera of mono-sensitized fish-allergic patients is a core requirement, which was a big hurdle for us. Another limitation is that the detailed clinical features of the patients enrolled were unavailable; the sIgE levels from the Phadia ImmunoCAP and the limited information about suspected culprit fish species were only available. Thus, we could not calculate exact sensitivity and specificity of the sIgE measurements with the new extracts. We compared the sIgE measurements of the investigatory extracts with those of the Phadia ImmunoCAP of the corresponding species and evaluated the correlations between them. We could only present the overall sensitivity

of investigatory extracts sIgE measurement for the identification of suspected causative fish species. As described above, however, the measurement of sIgE is not sufficient for the identification of culprit fish species.[16,17]

In conclusion, there are marked differences in the protein concentration and PV content of commercial SPT fish extracts. The list of commercially available fish allergen SPT extracts does not include some of the commonly consumed fish species in Korea. We produced the 6 new fish extracts of fish that are commonly consumed in Korea and found that our extracts contained a sufficient amount of the major PVs and had acceptable allergenicity and stability. We also confirmed the presence of marked cross-reactivity among the fish species in sIgE measurement, suggesting the requirement of other direct diagnostic tools for the identification of culprit fish species.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIALS

Supplementary Table S1

Source of fish allergens

Click here to view

Supplementary Fig. S1

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the new fish extracts stored in normal saline at room temperature (A-F) and 4°C (G-L) for up to a year.

Click here to view

Supplementary Fig. S2

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the new fish extracts stored in saline with 50% glycerol at room temperature (A-F) and 4°C (G-L) for up to a year.

Click here to view

Supplementary Fig. S3

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the new fish extracts stored in saline with 0.3% phenol at room temperature (A-F) and 4°C (G-L) for up to a year.

Click here to view

Supplementary Fig. S4

Anti-PV IgG immunoblotting of the new fish extracts stored in normal saline at room temperature (A-F) and 4°C (G-L) for up to a year.

Click here to view

Supplementary Fig. S5

Anti-PV IgG immunoblotting of the new fish extracts stored in saline with 50% glycerol at room temperature (A-F) and 4°C (G-L) for up to a year.

Click here to view

Supplementary Fig. S6

Anti-parvalbumin immunoglobulin G immunoblotting of the new fish extracts stored in saline with 0.3% phenol at room temperature (A-F) and 4°C (G-L) for up to a year.

Click here to view

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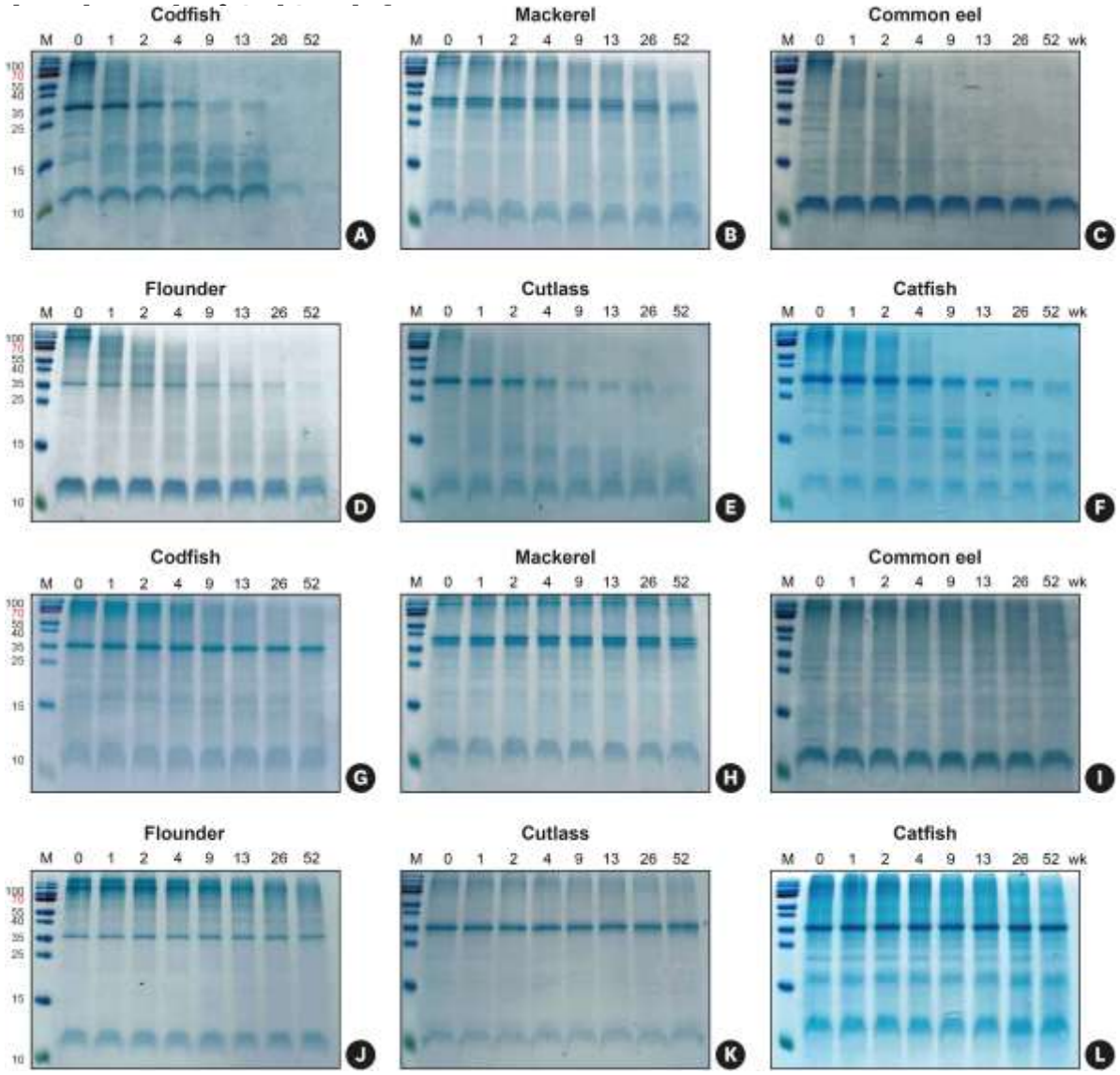
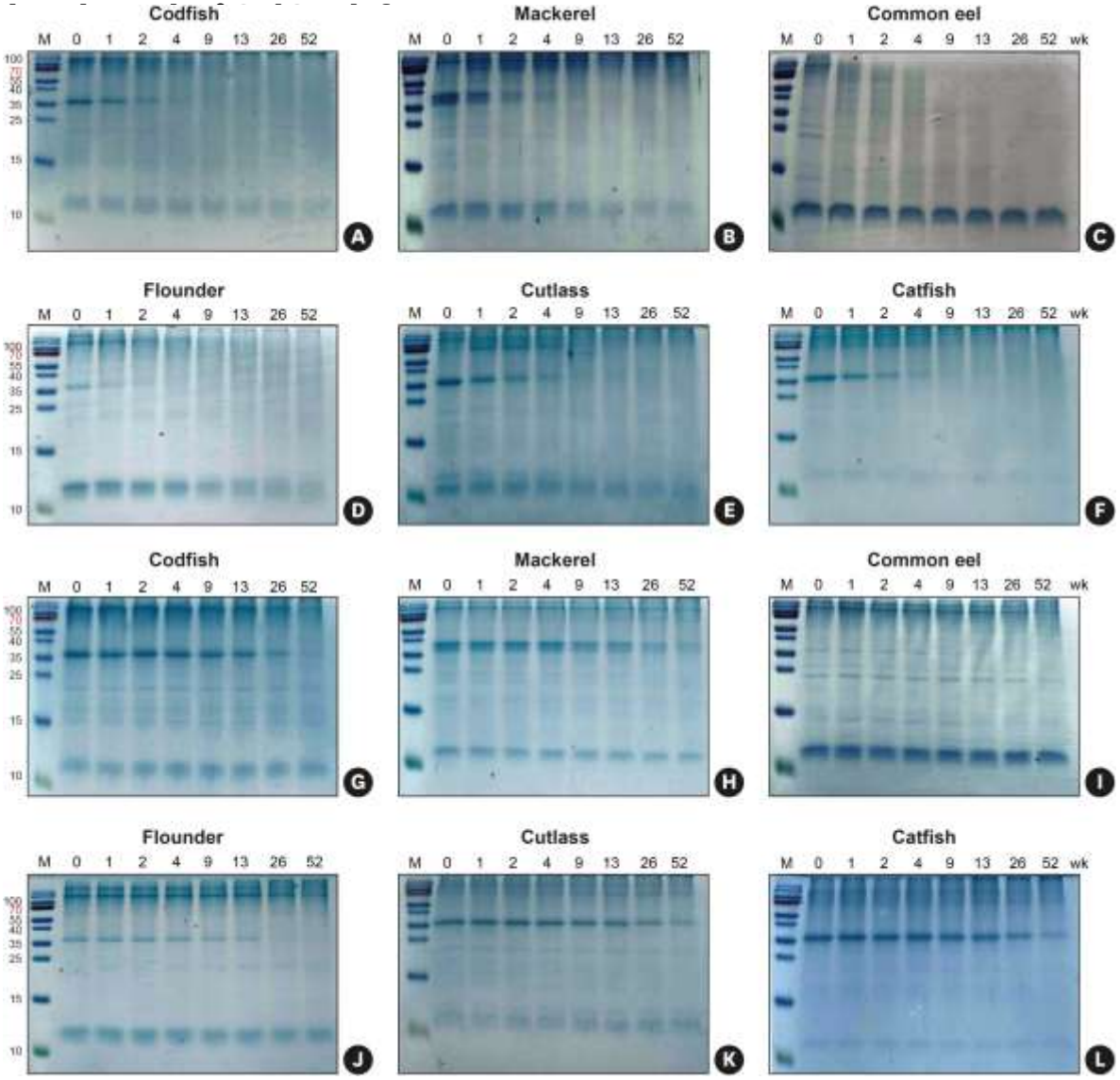
Supplementary Fig. S1. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the new fish extracts stored in normal saline at room temperature



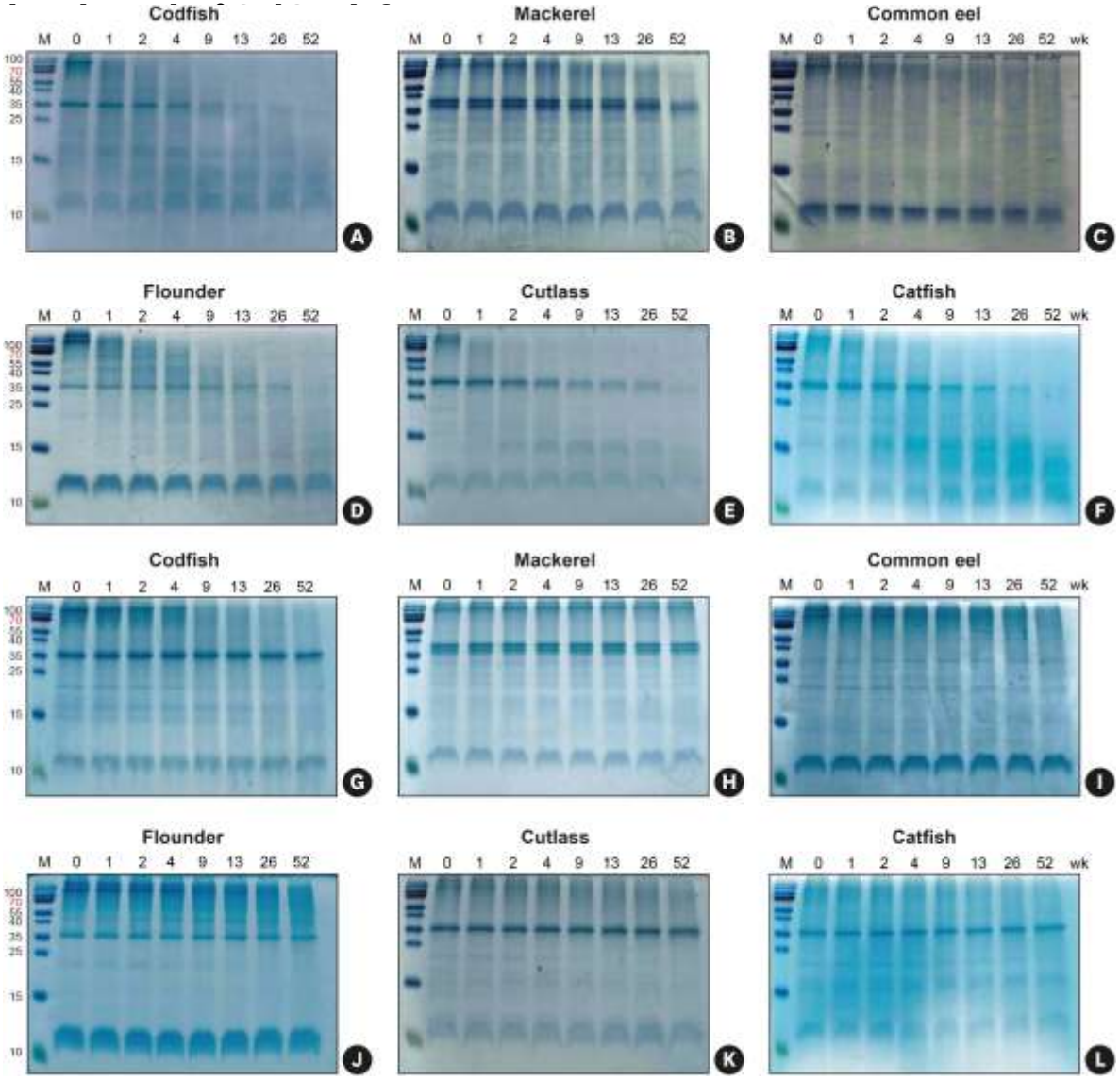
Allergy Asthma Immunol Res 2021 Jul;13(4):623-637. https://doi.org/10.4168/aair.2021.13.4.623

Supplementary Fig. S2. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the new fish extracts stored in saline with 50% glycerol at room temperature



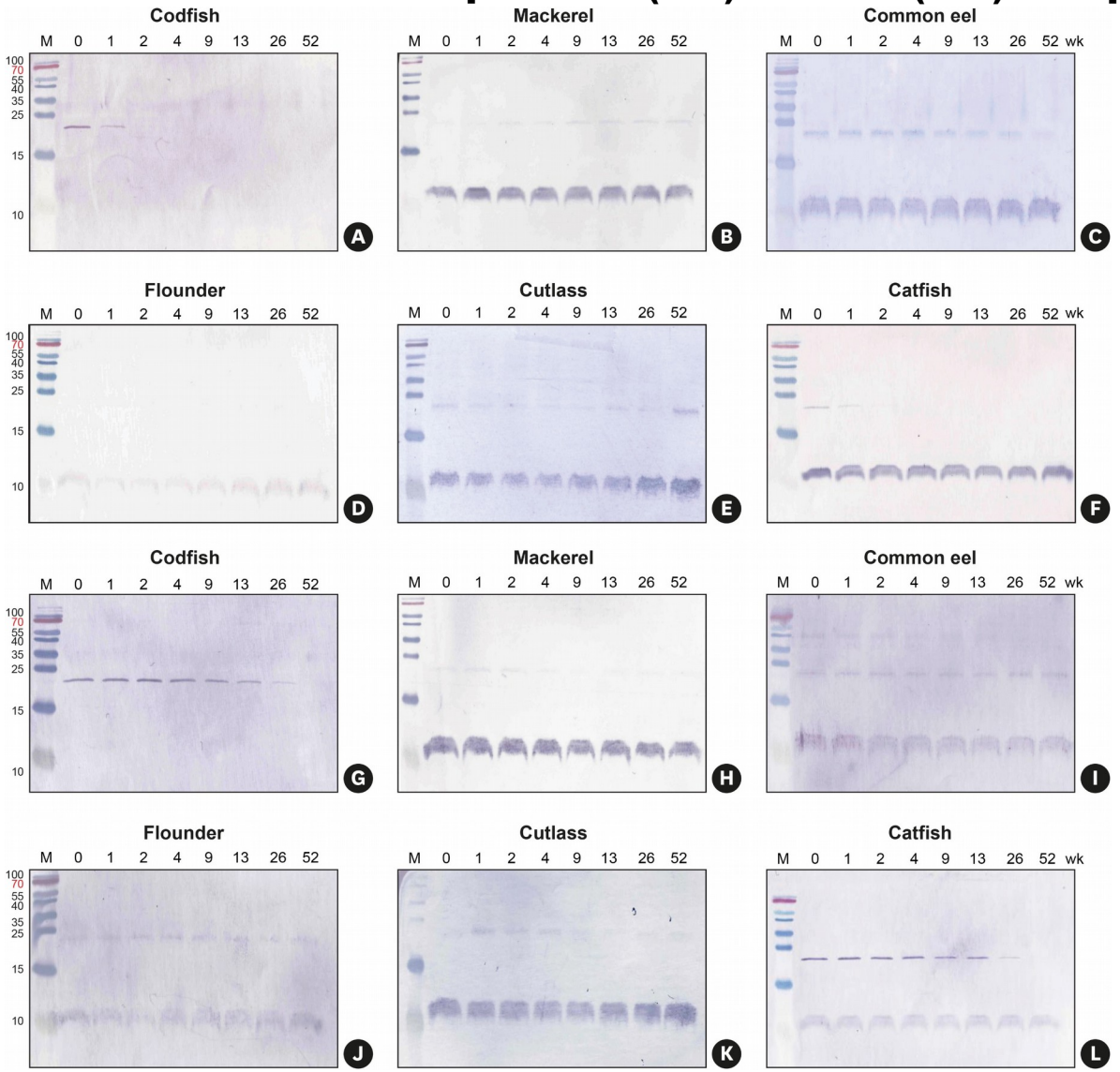
Allergy Asthma Immunol Res 2021 Jul;13(4):623-637. https://doi.org/10.4168/aair.2021.13.4.623

Supplementary Fig. S3. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the new fish extracts stored in saline with 0.3% phenol at room temperature



Allergy Asthma Immunol Res 2021 Jul;13(4):623-637. https://doi.org/10.4168/aair.2021.13.4.623

Supplementary Fig. S4. Anti-PV IgG immunoblotting of the new fish extracts stored in normal saline at room temperature (A-F) and 4°C (G-L) for up to a year.



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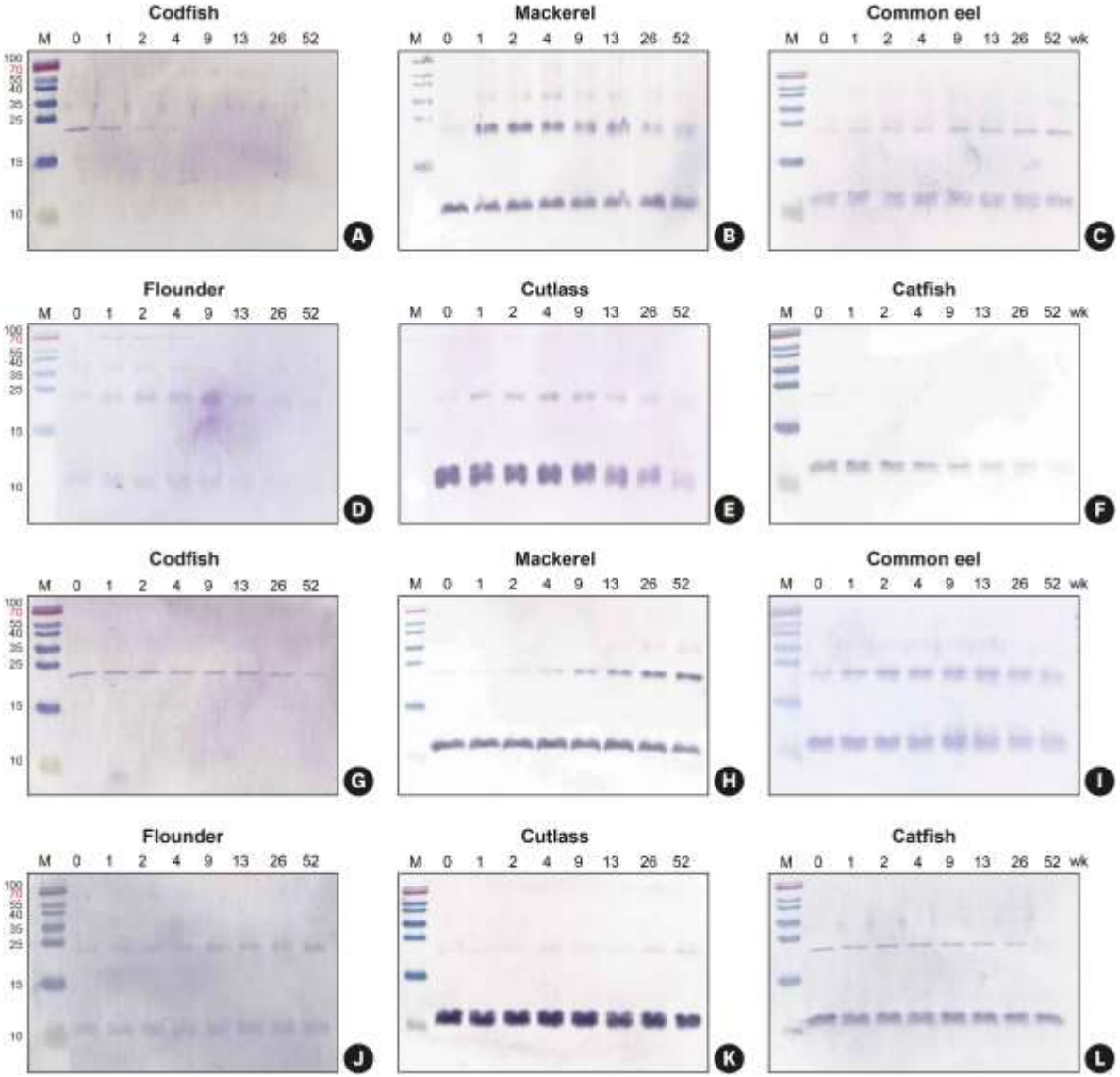
Supplementary Fig. S5. Anti-PV IgG immunoblotting of the new fish extracts stored in saline with 50% glycerol at room temperature (A-F) and 4°C (G-L) for up to a year.



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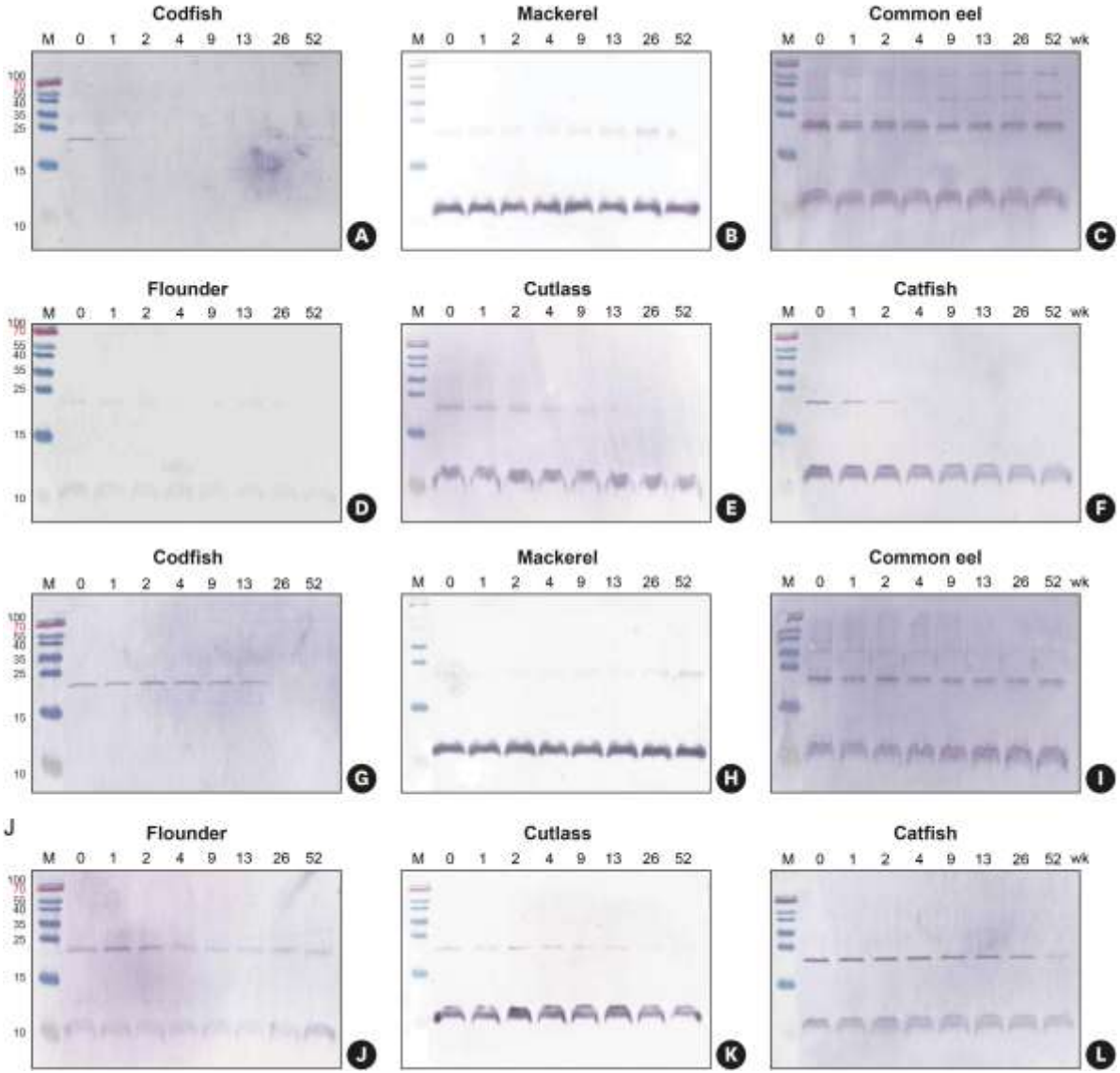
Supplementary Fig. S6. Anti-parvalbumin immunoglobulin G immunoblotting of the new fish extracts stored in saline with 0.3% phenol at room temperature (A-F) and 4°C (G-L) for



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