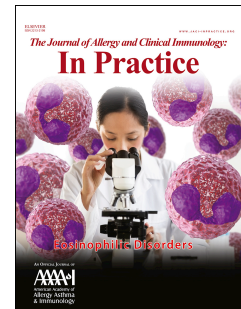


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Characteristics of Chinese fish-allergic patients: findings from double-blind placebo-controlled food challenges

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Characteristics of Chinese fish-allergic patients: findings from double-blind placebo-controlled food challenges

Running title: DBPCFCs in Chinese fish-allergic patients

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26 **Keywords:** Double-blind placebo-controlled food challenge; oral food challenge; fish allergy;
27 anaphylaxis; allergen component; component-resolved diagnosis; cod; carp; salmon; parvalbumin

28
29 **Conflict of interest:** All authors do not have any conflict of interest to declare

30
31
32 **Clinical Implications:**
33 Partial tolerance to selective fish species is common in Chinese fish-allergic patients. Choice of
34 fish allergens for sIgE measurement should be tailored to individual fish-allergic patients.

TO THE EDITOR:

The use of fish extracts in diagnosis is often held back by low specificity (1), varying allergen content in fish extracts (2) and the presence of cross-reactive hypoallergenic parvalbumin isoforms (3). Use of component-resolved diagnostic with Gad c 1 as the generic fish allergy marker overlooks the varying pattern of fish consumption across continents. Perez-gordo et al. (4) showed that sole and whiff parvalbumins were the major fish allergens in a Spanish population. Barramundi, salmon and tuna were the major causative species of fish allergy in Australia (5), while mackerel scad and anchovy were major culprits in the Philippines (6). Chinese culture and dietary habits are distinct, in which a recent survey (7) showed that grass carp was the top fish species consumed in Hong Kong children (25.6 g per week) whereas consumption of codfish was only 12.3 g per week. Our study aimed to evaluate the clinical characteristics of Chinese fish-allergic patients by the gold standard DBPCFCs and diagnostic performance of sIgEs to fish allergens.

Twenty-two subjects who exhibited clear history of immediate allergic reactions within two hours of fish consumption or contact on at least two exposures to fish in the past 2 years were analysed in the study (Figure E1). Clinical characteristics of our subjects are described in Table 1. For subjects who recalled the culprit fish species that induced allergic reactions, the highest frequency was grass carp followed by salmon and grouper, of which 75% had freshwater fish as the first allergic fish.

All subjects were offered oral, titrated challenges with grass carp, salmon and placebo on three separate days in randomized order, each of at least 48 hours apart (Dosage tables, blinding efficiency and recipes as shown in Table E4-6; photo of burger patties as shown in Figure E2). Eighty-one challenges were conducted in accordance to the PRACTALL consensus report (8) in 28 subjects: 25 subjects undergoing 3 DBPCFCs, 2 subjects undergoing salmon and placebo DBPCFCs (1 reported tolerance to grass carp and 1 had grass carp class 4 sIgE), and 1 subject

undergoing grass carp and placebo DBPCFCs (reported tolerance to salmon). Twenty subjects (71%) were challenge-positive, either to grass carp or salmon, and 8 subjects (29%) were challenge-negative for both grass carp and salmon (Table E2 & E3). Among the positive challenges, 14 subjects were positive to grass carp only, 1 subject was positive to salmon only, and 5 subjects were positive to both grass carp and salmon. The cumulative dose of carp to which those reacting in challenge varied from 0.27 to 80 g (median dose: 10 g), and that of salmon varied from 10 to 125 g (median dose: 52.8 g) (Figure E4). The most commonly observed symptoms during the DBPCFCs were mucocutaneous symptoms, predominantly peri-oral erythema, followed by itchy mouth or throat in the challenge-positive subjects (Table E7). Grass carp was the eliciting fish species in all subjects with severity score above 3 (9).

All subjects underwent skin prick test with fish mix and salmon extracts (ALK-Abello). The skin prick test (SPT) mean wheal diameters (MWDs) for fish mix, but not salmon extract, were significantly higher among grass carp challenge-positive than challenge-negative subjects (Tables E1a & b). However, the SPT MWDs of both fish mix and salmon extract did not differ significantly among the salmon challenge-positive and challenge-negative subjects.

The levels of sIgE to rGad c 1 and rCyp c 1, as measured by ImmunoCAP (Phadia/Thermo Fisher Scientific, Uppsala, Sweden), were significantly higher among grass carp challenge-positive subjects than challenge-negative subjects (Table E1a, Figures E3c-d). The sIgE to total IgE ratios of rGad c 1 and rCyp c 1 were also significantly higher among grass carp challenge-positive subjects (Table E1a). To a lesser extent, levels of sIgE to cod extract, but not the sIgE to total IgE ratio, were higher among challenge-positive subjects than challenge-negative subjects. In contrast, both the levels of sIgE and sIgE to total IgE ratio of salmon extract did not differ significantly between the grass carp challenge-positive and negative subjects (Table E1a, Figures E3b). ROC analysis showed an AUC for sIgE to cod extract, salmon extract, rGad c 1 and rCyp c 1 for grass carp allergy diagnosis of 0.854, 0.701, 0.920 and 0.917 respectively (Table E8a, Figure 1a). The diagnostic performance of fish allergen components was generally better among all

allergen measurements. The optimal cut-off value calculated by the Youden index was 0.91 and 2.14 kUA/L for rGad c 1 and rCyp c 1 respectively.

Only the levels of sIgE and sIgE to total IgE ratio to salmon extract were significantly higher among salmon challenge-positive subjects than challenge-negative subjects (Table E1b, Figures E3b). ROC analysis showed an AUC for sIgE to cod extract, salmon extract, rGad c 1 and rCyp c 1 for salmon allergy diagnosis of 0.746, 0.881, 0.659 and 0.603 respectively (Table E8b, Figure 1b). As opposed to grass carp allergy where recombinant parvalbumins yielded better diagnostic performance, sIgE to salmon extract was a superior diagnostic marker for clinical salmon allergy. There was, however, no commercially available recombinant salmon parvalbumin for comparison and the number of salmon-allergic subjects in our study was too low to draw conclusions.

To the best of our knowledge, this study is the first report on the clinical characteristics and diagnostic performance of commercially available immunologic markers for fish allergy among challenge-confirmed patients. In our cohort, use of rGad c 1 and rCyp c 1 yielded the highest area under the receiver operating characteristic curves (AUC) of 0.920 and 0.917 in grass carp-allergic subjects, whereas our preliminary results suggest that optimal detection of salmon allergy requires salmon extract.

We concluded that the majority of our study population was found to be partially tolerant to selective fish species by DBPCFCs. Choice of fish allergens for sIgE measurement should be tailored to individual fish-allergic patients. Recombinant parvalbumins to cod and carp have improved diagnostic performance in grass carp-allergic subjects but presumably not in salmon-allergic subjects. Further research on component-resolved diagnosis in fish allergy is warranted.

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Table 1 – Comparison of patient characteristics in the OFC-positive and OFC-negative groups

Results of fish challenges	Total	Positive	Negative	p-value
Number of patients	28	20 (Carp only = 14 Salmon only = 1 Carp & salmon = 5)	8	N/A
Sex, no. of boys (%)	18 (64.3)	11 (55)	7 (87.5)	0.105
Average age at challenge, median, years (min, max)	6.9 (2.0, 21.6)	7.9 (2.0, 21.6)	5.2 (2.3, 15.5)	0.615
Age at first fish-allergic reactions, median, months (min, max)	8 (6, 36)	8 (6, 36)	9 (6, 18)	0.903
Time from 1 st fish-allergic reaction to age at challenge, median, years (min, max)	5.3 (1.0, 20.6)	6.8 (1.0, 20.6)	4.7 (1.8, 14.6)	0.686
History of fish-induced anaphylaxis (%)	5 (17.9)	5 (25)	0 (0)	0.119
Freshwater fish as first allergic fish, no./total (%)	18/24 (75)	14/20 (70)	4/8 (50)	0.318
Comorbidities – atopic dermatitis (%)	28 (100)	100 (20)	8 (100)	N.A.
asthma (%)	12 (42.9)	8 (40)	4 (50)	0.629
allergic rhinitis (%)	22 (78.6)	15 (75)	7 (87.5)	0.466
Other food allergies (%)	15 (53.6)	12 (60)	3 (37.5)	0.281

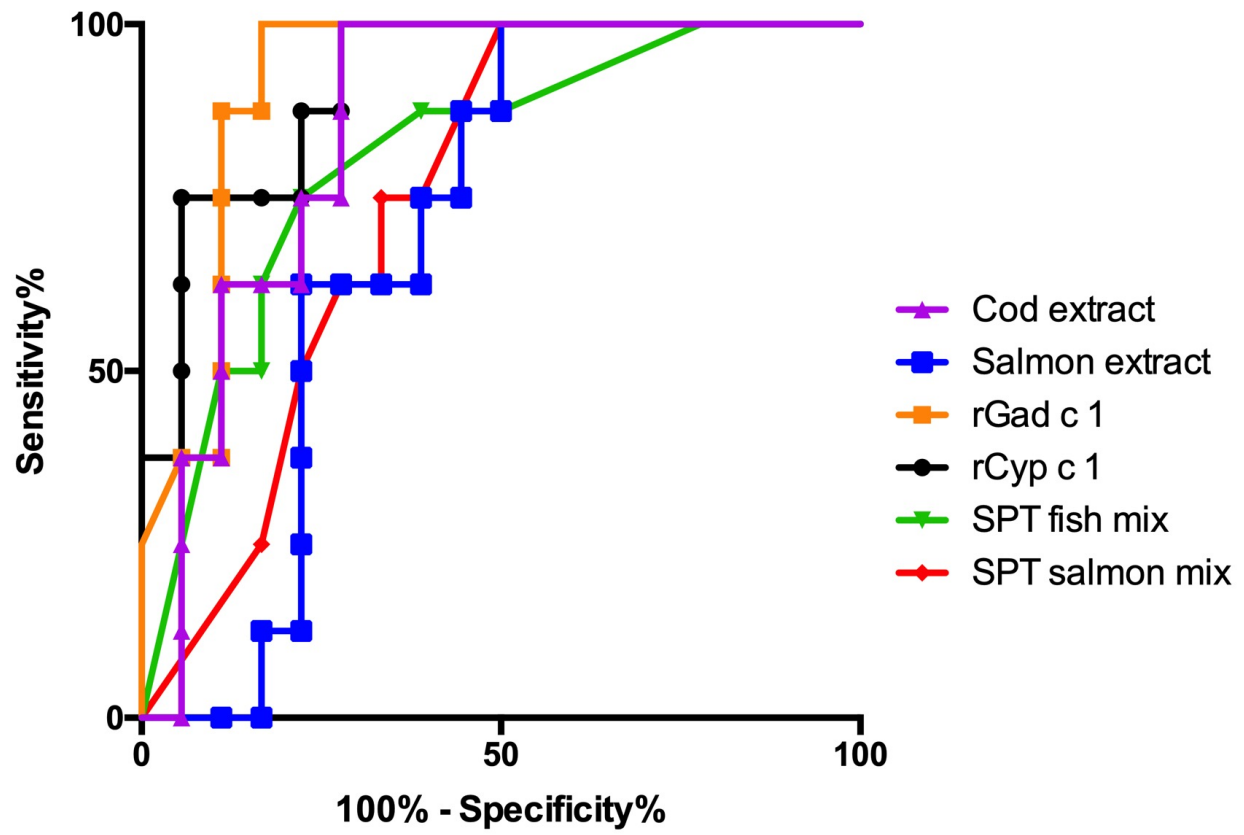
kUA /L, Kilounits of allergen-specific IgE per litre; PV, parvalbumin, no., number

Figure Legends

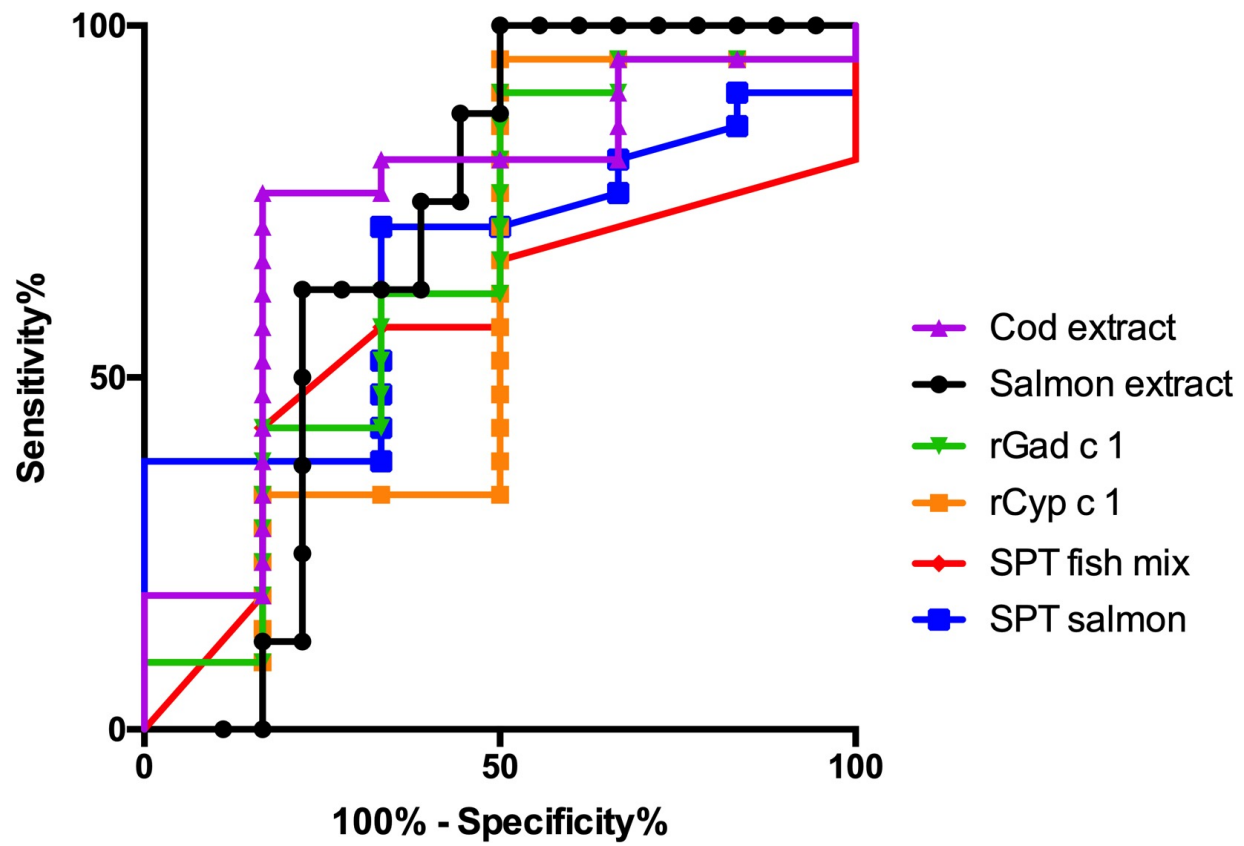
Figure 1a: Diagnostic performance of sIgE and SPT for grass carp allergy diagnosis, showing the receiver operating characteristic curves for sIgE levels to cod extract, salmon extract, rGad c 1 and rCyp c 1, and SPT to fish mix and salmon. Area under the receiver operating characteristic curves (AUC) was 0.854, 0.701, 0.920, 0.917, 0.809 and 0.747 respectively. Results are based on ImmunoCAP results of sera from challenge-positive and challenge-negative study participants, and meal wheal diameters of the skin prick test results to fish mix and salmon. Purple, blue, orange, black, green and red curves represent receiver operating curves of cod extract, salmon extract, rGad c 1, rCyp c 1, SPT to fish mix and SPT to salmon respectively.

Figure 1b: Diagnostic performance of sIgE and SPT for salmon allergy diagnosis, showing the receiver operating characteristic curves for sIgE levels to cod extract, salmon extract, rGad c 1 and rCyp c 1, and SPT to fish mix and salmon. Area under the receiver operating characteristic curves (AUC) was 0.746, 0.881, 0.659, 0.603, 0.564 and 0.659 respectively. Results are based on ImmunoCAP results of sera from challenge-positive and challenge-negative study participants, and meal wheal diameters of the skin prick test results to fish mix and salmon. Purple, black, green, orange, red and blue curves represent receiver operating curves of cod extract, salmon extract, rGad c 1, rCyp c 1, SPT to fish mix and SPT to salmon respectively.

ROC curves for grass carp allergy diagnosis



ROC curves for salmon allergy diagnosis



Patients who exhibited a clear history of immediate allergic reaction within 2 hours of fish consumption or contact on at least 2 exposures to fish
n=50

Excluded due to:

- Illness on the day of OFC (n=1)
- Severe/uncontrolled atopic dermatitis (n=3)
- High specific IgE antibodies ≥ 17.5 kUA/L (class 4) to either cod or salmon (n=7)
- History of anaphylaxis (n=3)
- Subjects/ parents refused OFC (n=8)

Patients included in double-blind placebo-controlled food challenges

- Positive to SPT against fish mix or salmon extracts
- Specific IgE antibodies ≥ 0.35 kUA/L to cod or salmon

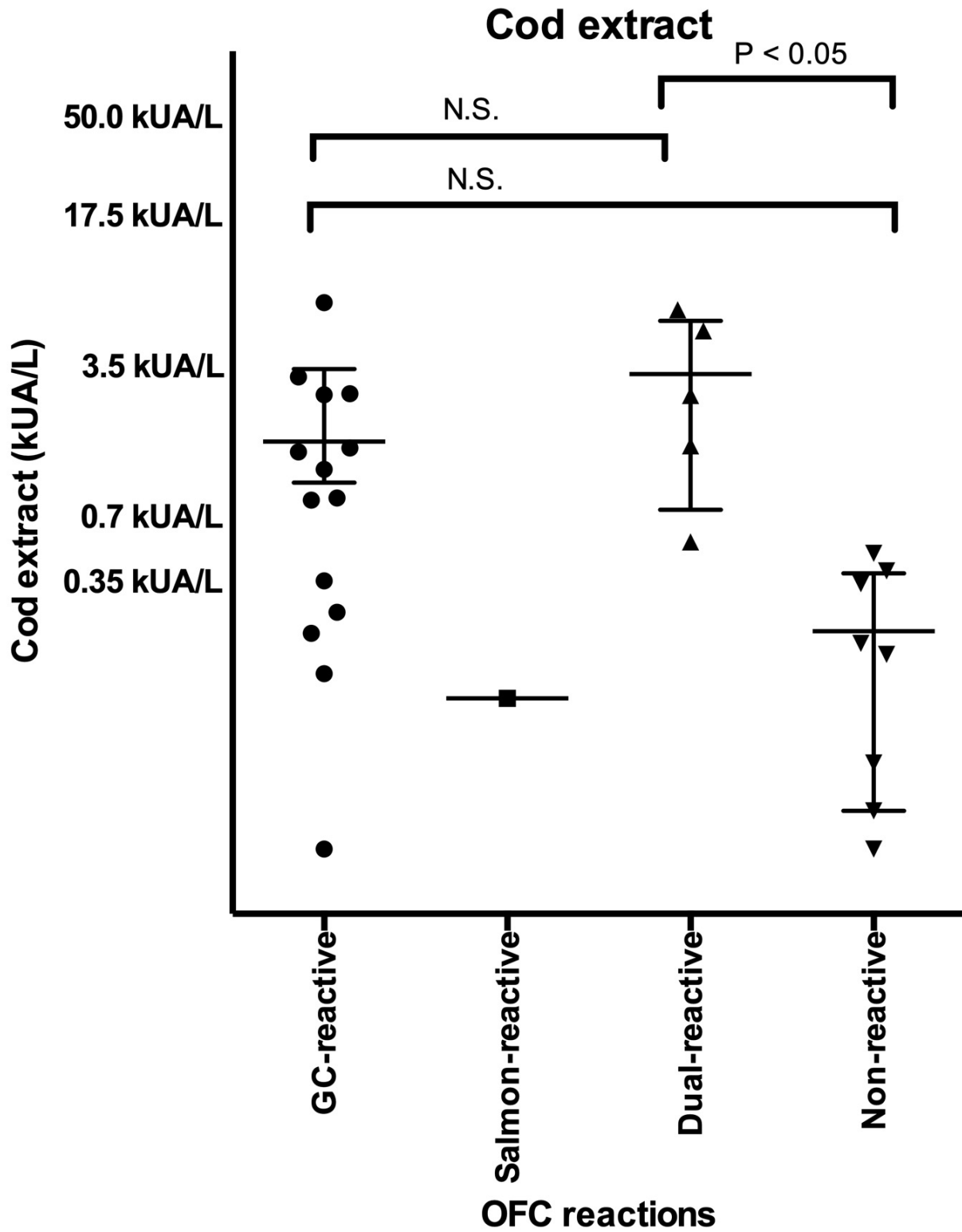
n=28

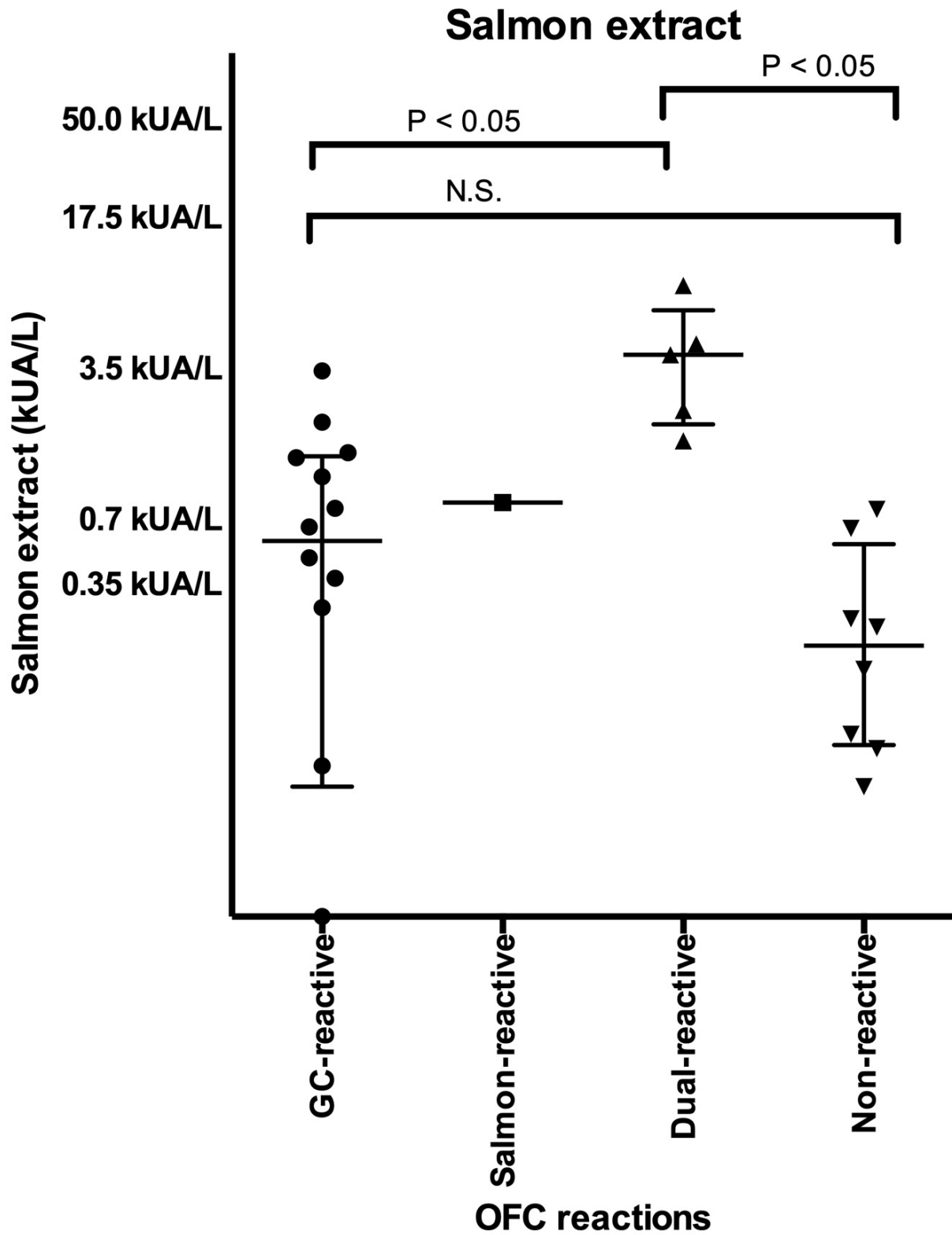
Grass carp +
salmon + placebo
DBPCFCs
n=25

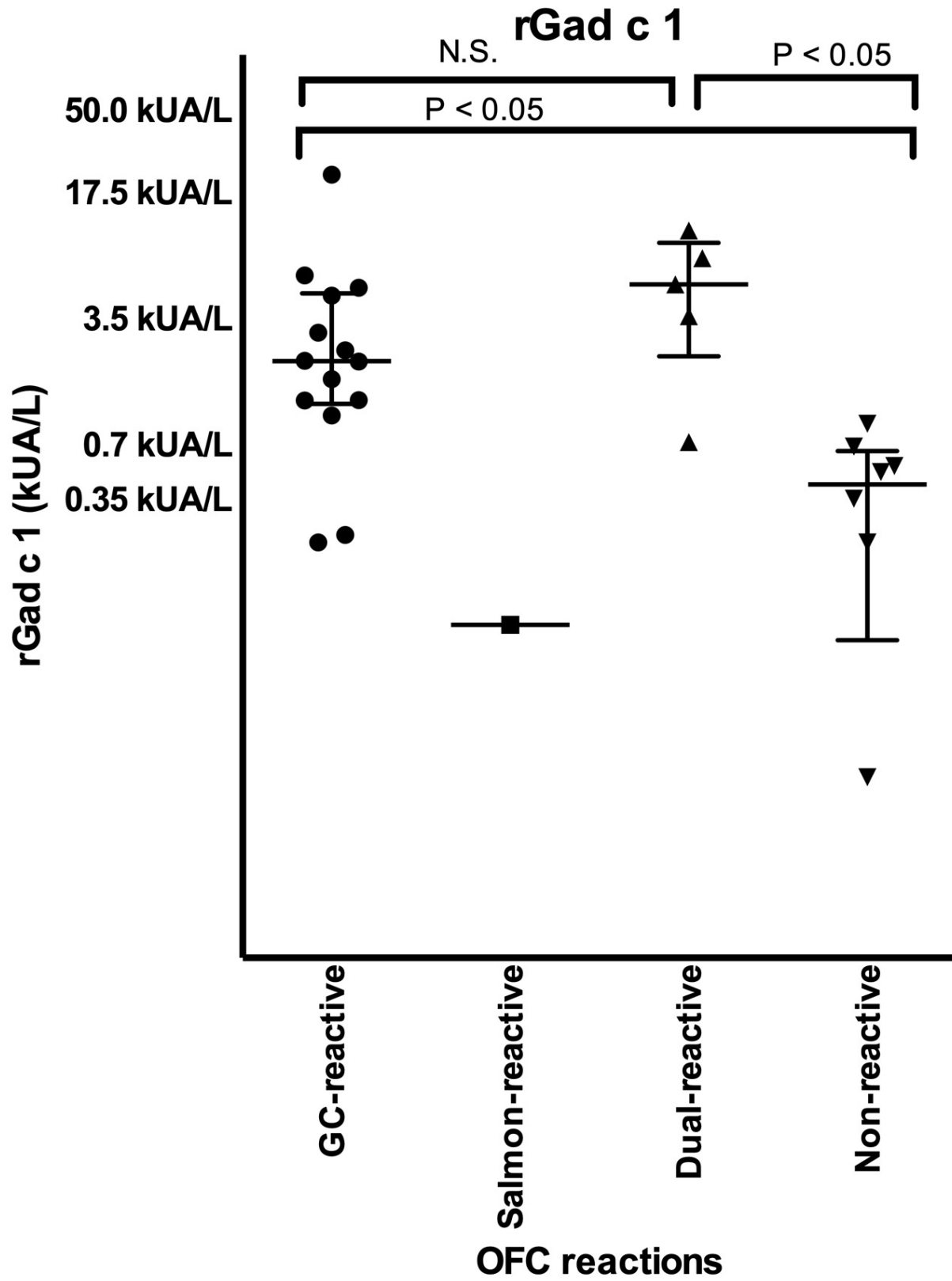
Grass carp +
placebo
DBPCFCs
n=1

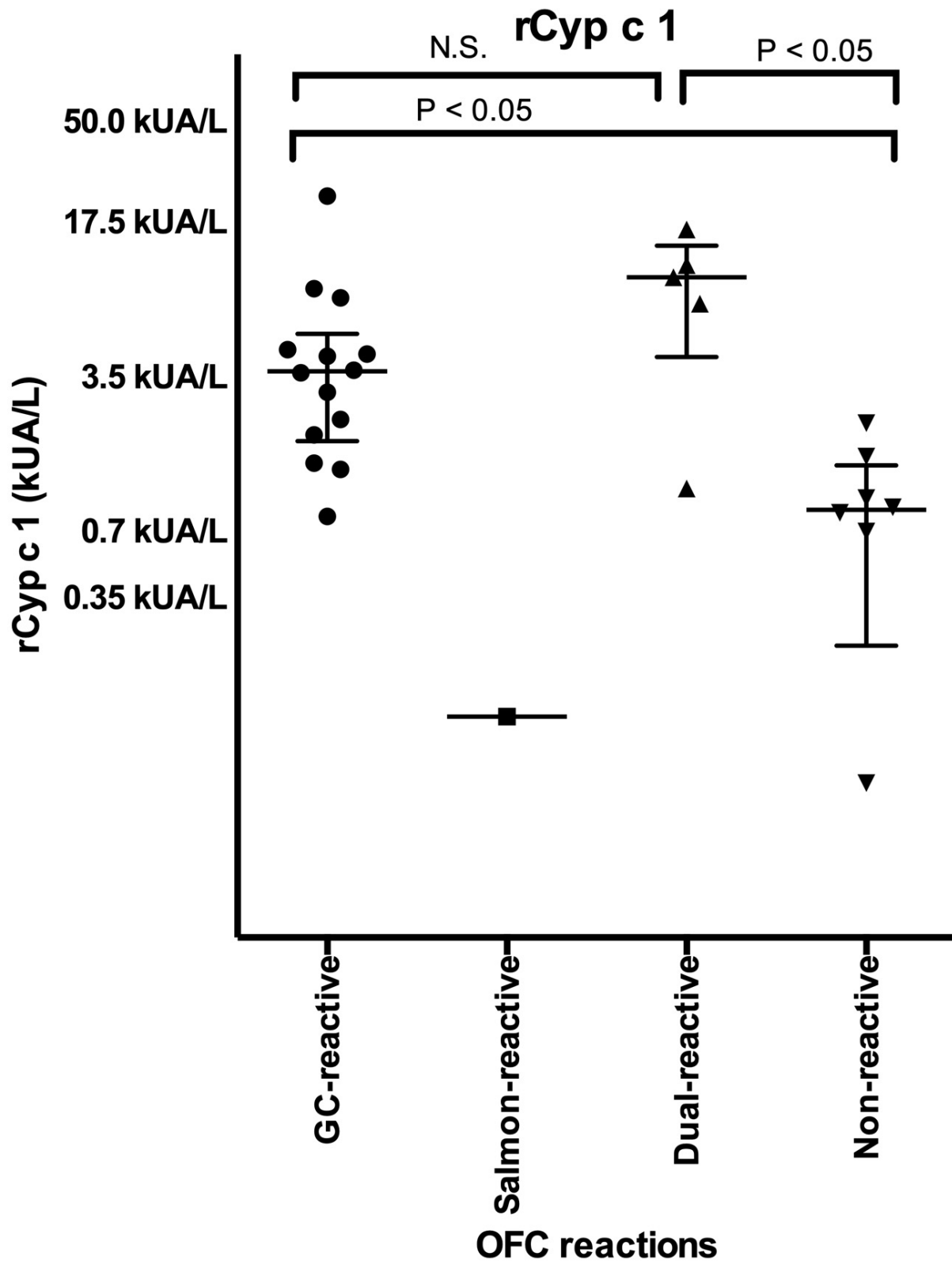
Salmon +
placebo
DBPCFCs
n=2

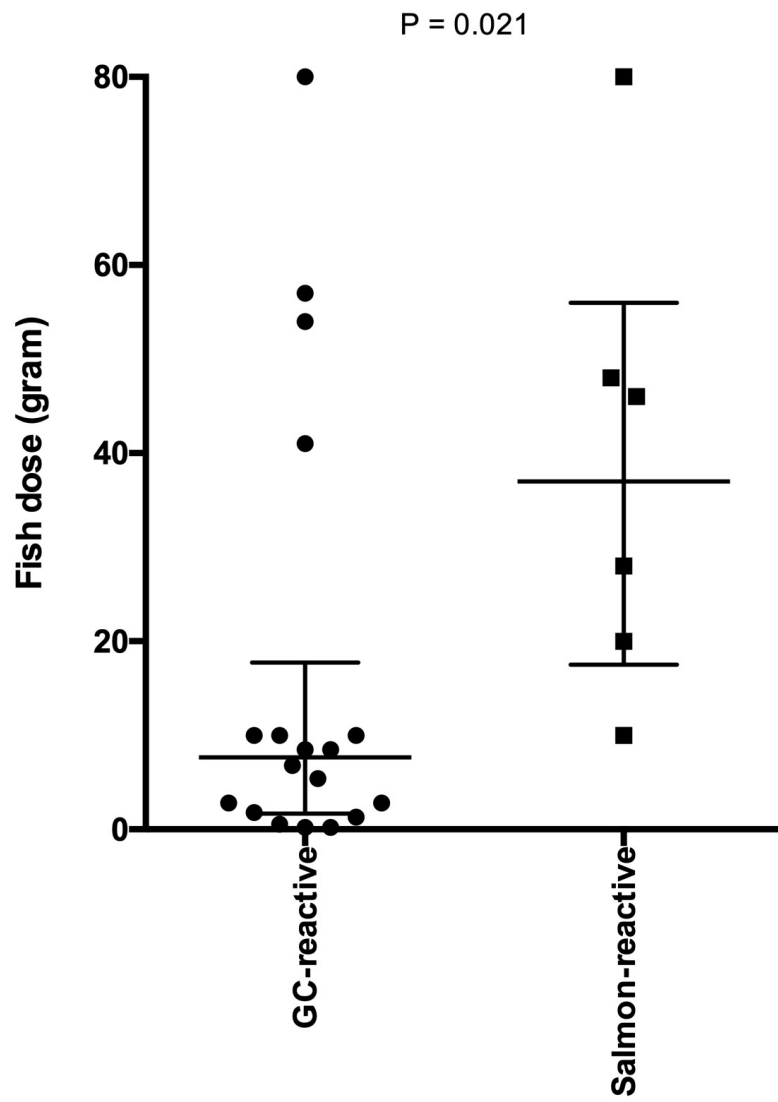












LOAELs in challenge-positive subjects

Table E1a – Comparison of immunological test results in the **grass carp-challenge** positive and negative groups

Results of fish challenges	Total (n=26)	Positive (n=18)	Negative (n=8)	p-value
SPT – fish mix, median, wheal in mm (IQR)	5 (3.13 - 7)	6.5 (5 - 7)	1.75 (0 - 4.25)	0.011
SPT – fish mix, no. of MWD $\geq$ 3mm (%)	20 (76.9)	16 (88.9)	4 (50)	0.030
SPT – salmon, median, wheal in mm (IQR)	4.75 (2 - 6.38)	5.3 (3.13 - 7)	2.5 (1.5 - 4.25)	0.048
SPT – salmon, no. of MWD $\geq$ 3mm (%)	18 (69.2)	14 (77.8)	4 (50)	0.157
Cod extract sIgE, median, kUA/L (IQR)	0.50 (0.19 - 1.47)	1.27 (0.4 - 2.56)	0.17 (0.09 - 0.35)	0.003
Cod extract *sIgE to total IgE ratio (IQR)	0.183 (0.041 - 0.38)	0.274 (0.071 - 0.414)	0.024 (0.019 - 0.325)	0.557
No. of Cod extract sIgE $\geq$ 0.35 kUA/L (%)	16 (61.5)	14 (77.8)	2 (25)	0.011
Salmon extract sIgE, median, kUA/L (IQR)	0.64 (0.16 - 1.39)	0.935 (0.3 - 1.86)	0.23 (0.123 - 0.665)	0.112
Salmon extract sIgE to total IgE ratio (IQR)	0.076 (0.02 - 0.33)	0.105 (0.05 - 0.291)	0.027 (0.013 - 0.522)	0.838
No. of Salmon extract sIgE $\geq$ 0.35 kUA/L (%)	16 (61.5)	13 (72.2)	3 (37.5)	0.093
Cod PV (rGad c 1), median, kUA/L (IQR)	1.23 (0.5 - 3.4)	2.18 (1.22 - 5.02)	0.42 (0.17 - 0.57)	<0.001
Cod PV sIgE to total IgE ratio (IQR)	0.337 (0.119 - 0.622)	0.410 (0.256 - 0.726)	0.073 (0.032 - 0.343)	0.040
No. of rGad c 1 sIgE $\geq$ 0.35 kUA/L (%)	21 (80.8)	16 (88.9)	5 (62.5)	0.115
Common Carp PV (rCyp c 1), median, kUA/L (IQR)	2.18 (1.01 - 4.56)	4.01 (1.97 - 7.79)	0.87 (0.54 - 1.11)	<0.001
Common Carp PV sIgE to total IgE ratio (IQR)	0.543 (0.249 - 1.1)	0.618 (0.432 - 1.267)	0.146 (0.102 - 0.437)	0.022
No. of rCyp c 1 sIgE $\geq$ 0.35 kUA/L (%)	24 (92.3)	18 (100)	6 (75)	0.027

MWD, mean wheal diameter; no., number; kUA/L, Kilounits of allergen-specific IgE per litre; PV, parvalbumin; IQR, interquartile range

*sIgE to total IgE ratio was calculated by (sIgE/tIgE) x 100

Table E1b – Comparison of immunological test results in the **salmon-challenge** positive and negative groups

Results of fish challenges	Total (n=27)	Positive (n=6)	Negative (n=21)	p-value
SPT – fish mix, median, wheal in mm (IQR)	5 (3.25 - 7)	6.25 (5.13 - 7)	5 (3 - 7)	0.657
SPT – fish mix, no. of MWD ≥ 3 mm (%)	22 (81.5)	5 (83.3)	17 (81.0)	0.895
SPT – salmon, median, wheal in mm (IQR)	5 (2 - 6.25)	5.75 (3.63 - 6.75)	4.5 (2 - 6)	0.254
SPT – salmon, no. of MWD ≥ 3 mm (%)	19 (70.4)	6 (100)	13 (61.9)	0.072
Cod extract sIgE, median, kUA/L (IQR)	0.53 (0.19 - 2.01)	2.01 (1.41 - 4.43)	0.39 (0.18 - 1.15)	0.073
Cod extract *sIgE to total IgE ratio (IQR)	0.153 (0.033 - 0.376)	0.386 (0.252 - 0.419)	0.105 (0.025 - 0.366)	0.059
No. of Cod extract sIgE ≥ 0.35 kUA/L (%)	17 (63.0)	5 (83.3)	12 (57.1)	0.241
Salmon extract sIgE, median, kUA/L (IQR)	0.64 (0.18 - 1.51)	3.12 (1.18 - 4.38)	0.37 (0.07 - 1.09)	0.003
Salmon extract sIgE to total IgE ratio (IQR)	0.082 (0.027 - 0.357)	0.357 (0.224 - 0.678)	0.057 (0.021 - 0.211)	0.018
No. of Salmon extract sIgE ≥ 0.35 kUA/L (%)	17 (63.0)	6 (100)	11 (52.4)	0.033
Cod PV (rGad c 1), median, kUA/L (IQR)	1.22 (0.42 - 4.12)	3.69 (1.04 - 6.97)	1.01 (0.35 - 2.9)	0.259
Cod PV sIgE to total IgE ratio (IQR)	0.315 (0.084 - 0.616)	0.473 (0.282 - 0.618)	0.287 (0.066 - 0.603)	0.550
No. of rGad c 1 sIgE ≥ 0.35 kUA/L (%)	21 (77.8)	5 (83.3)	16 (76.2)	0.711
Common Carp PV (rCyp c 1), median, kUA/L (IQR)	2.14 (0.94 - 6.03)	5.56 (1.14 - 10.77)	2.14 (0.89 - 4.39)	0.476
Common Carp PV sIgE to total IgE ratio (IQR)	0.542 (0.214 - 1.045)	0.464 (0.371 - 0.832)	0.544 (0.184 - 1.162)	0.942
No. of rCyp c 1 sIgE ≥ 0.35 kUA/L (%)	24 (88.9)	5 (83.3)	19 (90.5)	0.623

kUA/L, Kilounits of allergen-specific IgE per litre; PV, parvalbumin; IQR, interquartile range

*sIgE to total IgE ratio was calculated by (sIgE/tIgE) x 100

Table E2 – Sex, age, wheal size of skin prick test, sIgE to fish allergens, other allergic diseases, positive results of double-blinded, placebo-controlled food challenge (DBPCFC) per subject followed by ED, CD and symptoms severity score - characteristics of the **20 subjects with 25 positive DBPCFC reactions** (14 subjects were positive to grass carp only, 1 positive to salmon only, and 5 positive to both grass carp and salmon)

Pt no	Sex	Age (year)	Ag	Total IgE (kU/L)	SPT fish (mm)	SPT salmon (mm)	Cod extract sIgE (kUA/L)	Salmon extract sIgE (kUA/L)	Cod PV (rGad c 1) sIgE (kUA/L)	Common Carp PV (rCyp c 1) sIgE (kUA/L)	Other allergic diseases	Symptoms	ED (g)	CD (g)	Score
1	F	14	carp	946	6	5	1.45	0.78	2.32	4.61	AS, AD, AR	GI, S, R	57	57	4
2	F	4	carp	285	7	6	2.54	2.22	5.36	9.79	AD, AR	GI, S	5.4	7.98	1
2	F	4	salmon	285	7	6	2.54	2.22	5.36	9.79	AD, AR	GI, S	80	80	1
3	F	21	carp	2482	7	3	1.48	8.38	0.72	1.08	AD, AR	GI, S, R	2.8	4.02	3
3	F	21	salmon	2482	7	3	1.48	8.38	0.72	1.08	AD, AR	GI, S	28	40.52	1
4	M	11	carp	343	7	9	1.15	1.09	2.03	2.22	AS, AD, AR	GI, S	8.5	12.52	1
5	M	6	carp	720	5	2	0.35	0.05	1.23	1.89	AD, AR	GI, S	10	10	3
6	F	5	carp	668	5	7.5	2.56	1.41	5.14	8.7	AS, AD, AR	GI, S	1.8	2.58	3
7	M	3	salmon	27.3	0	3	0.10	0.83	0.07	0.1	AD	GI, S	46	46	3
8	M	11	carp	614	4	3.5	3.09	0.27	4.66	3.62	AD, AR	GI, S, R	8.5	12.52	4
9	M	10	carp	391	6	6	6.84	3.38	21.7	22.8	AS, AD, AR	GI, S*	/	/	3
10	F	9	carp	69.7	11	4.5	0.02	0	0.2	0.81	AD, AR	GI, S, CNS	0.2	0.27	3
11	M	3	carp	1913	9	5	2.58	1.34	6.02	7.9	AD, AR	GI, S	54	80	1
12	F	3	carp	47.9	3	0	0.20	0.01	0.22	1.41	AD	GI, S	80	80	1
13	M	2	carp	28.4	0	0	0.13	0	1.6	2.95	AD	GI, S	1.3	1.88	1
14	M	13	carp	1522	7.5	7	0.83	1.95	1.01	3.72	AS, AD, AR	GI, S	10	10	1
15	F	15	carp	2968	7	8	6.34	4.01	10.7	16.1	AS, AD, AR	GI, S	10	10	3
15	F	15	salmon	2968	7	8	6.34	4.01	10.7	16.1	AS, AD, AR	GI, S	10	10	3
16	F	7	carp	1195	5	7	5.06	4.50	7.51	11.1	AS, AD, AR	GI, S	6.8	9.97	3
16	F	7	salmon	1195	5	7	5.06	4.50	7.51	11.1	AS, AD, AR	GI, S	20	20	3
17	F	15	carp	343	5.5	5.5	1.39	0.64	2.01	1.32	AD, AR	GI, S	2.8	4.02	3
18	M	8	carp	807	9	9	0.85	0.46	2.9	4.39	AS, AD, AR	GI, S, R	0.2	0.27	4
19	M	4	carp	145	7	6.5	0.53	1.60	3.57	7.44	AD	GI, S	0.54	0.78	3
19	M	4	salmon	145	7	6.5	0.53	1.60	3.57	7.44	AD	GI, S	48	48	3
20	M	1	carp	654	0	0	0.25	0.37	1.22	4.3	AD	S	41	47.9	1

Pt no, patient number; Ag, allergen; SPT, skin prick test; sIgE, specific IgE; PV, parvalbumin; ED, eliciting dose; CD, cumulative dose; g, gram; F, female; M, male; AS, asthma; AD, atopic dermatitis; AR, allergic rhinitis; GI, gastrointestinal; S, skin; R, respiratory; CNS, central nervous system; kUA/L, Kilounits of allergen-specific IgE per litre.

Table E3 – Sex, age, wheal size of skin prick test, sIgE to fish allergens and other allergic diseases - characteristics of the 8 subjects with **negative** DBPCFC reactions (negative to both grass carp and salmon)

Pt no	Sex	Age (year)	Ag	Total IgE (kU/L)	SPT fish (mm)	SPT salmon (mm)	Cod extract sIgE (kUA/L)	Salmon extract sIgE (kUA/L)	Cod PV (rGad c 1) sIgE (kUA/L)	Common Carp PV (rCyp c 1) sIgE (kUA/L)	Other allergic diseases
1	M	15	/	1674	7	2	0.39	0.22	0.53	1.51	AS, AD, AR
2	M	4	/	606	4	5	0.05	0.14	0.01	0.05	AS, AD, AR
3	F	4	/	92.1	0	2	0.34	0.77	0.68	2.14	AD, AR
4	M	14	/	641	3.5	4	0.16	0.07	0.2	0.69	AD, AR
5	M	4	/	190	3	1	0.02	0.06	0	0	AS, AD, AR
6	M	2	/	793	0	0	0.18	0.24	0.35	0.84	AD
7	M	6	/	484	5	5	0.03	0.04	0.49	0.89	AS, AD, AR
8	M	3	/	151	0	0	0.47	0.63	0.91	0.98	AD, AR

Pt no, patient number; Ag, allergen; SPT, skin prick test; sIgE, specific IgE; PV, parvalbumin; g, gram; F, female; M, male; AS, asthma; AD, atopic dermatitis; AR, allergic rhinitis; kUA/L, Kilounits of allergen-specific IgE per litre

Table E4 – The food challenge dosages according to four age groups

0-3 years old (A)				
Dose	Fish in g	Cumulative	Protein in g	Cumulative
1	0.04	0.04	0.007	0.007
2	0.13	0.17	0.02	0.027
3	0.41	0.58	0.07	0.097
4	1.3	1.88	0.22	0.317
5	4.1	5.98	0.68	0.997
6	13	18.98	2.17	3.167
7	41	59.98	6.84	10.007

3-6 years old (B)				
Dose	Fish in g	Cumulative	Protein in g	Cumulative
1	0.06	0.06	0.01	0.01
2	0.18	0.24	0.03	0.04
3	0.54	0.78	0.09	0.13
4	1.8	2.58	0.3	0.43
5	5.4	7.98	0.9	1.33
6	18	25.98	3	4.33
7	54	79.98	9	13.33

6-10 years old (C)				
Dose	Fish in g	Cumulative	Protein in g	Cumulative
1	0.07	0.07	0.01	0.01
2	0.2	0.27	0.04	0.05
3	0.7	0.97	0.1	0.15
4	2.2	3.17	0.4	0.55
5	6.8	9.97	1.1	1.65
6	22	31.97	3.6	5.25
7	68	99.97	11.3	16.55

10+ years old (D)				
Dose	Fish in g	Cumulative	Protein in g	Cumulative
1	0.09	0.09	0.02	0.02
2	0.28	0.37	0.05	0.07
3	0.85	1.22	0.14	0.21
4	2.8	4.02	0.47	0.68
5	8.5	12.52	1.42	2.1
6	28	40.52	4.68	6.78
7	85	125.52	14.2	20.98

Table E5 – Subjects who identified the active food sample (Correct Answer) and subjects who did not (Incorrect Answer)

Active food sample	Correct	Incorrect	Total	p-value
Grass carp	5	13	18	n.s.
Salmon	6	6	12	n.s.
Placebo	12	3	15	n.s.

n.s., non-significant ($p \geq .05$)

Table E6 – Recipe of the DBPCFC-burger patties prepared for *dose 7 of Group A subjects* (serves 4 patties)

Ingredients	
Fish fillet, cooked and deboned (for verum only)	30 grams
Minced pork	60 grams
Onion, roughly chopped	20 grams
Corn	3 tablespoons
Carrot, finely chopped	3 tablespoons
Oats	1 tablespoon
Corn starch	1 tablespoon
Mushroom powder	1/2 teaspoon
Paprika	1/4 teaspoon
White pepper	1/4 teaspoon
Black pepper	To taste
Sugar	1/4 teaspoon
Parsley	1/8 teaspoon
Water	1 tablespoon
Methods: 1. Blend the fish fillet, minced meat and chopped onion for 1 minute. 2. In a large mixing bowl, combine the blended mixture, corn, carrots, oats, corn starch and water. 3. Mix well and season with mushroom powder, paprika, white pepper, black pepper, sugar and parsley. 4. Pack burger patty individually by cling wrap, label clearly, and store in the designated freezer at -20°C. 5. Transfer the frozen burger patties from freezer to refrigerator (at 0-4°C) 12 hours before use. <i>On the day of OFC</i> 6. Take out the burger patties from the refrigerator 1 hour before use. 7. Heat a tablespoon of olive oil in a frying pan over a medium heat. 8. Add in the burger patties when temperature of pan reaches around 150°C. 9. Pan fry the patties for about 3 minutes on each side until golden. 10. Keep warm in aluminum foil.	

Table E7 – Induced symptoms and severity of the positive fish OFC test reactions

Characteristics	Total	Total (%)
Symptoms	Oral-mucosal manifestation	25 (100)
	Gastrointestinal manifestation	24 (96)
	Respiratory manifestation	4 (16)
	Central Nervous System manifestation	1 (4)
	Cardiovascular manifestation	0 (0)
Severity	Grade 1	9 (36)
	Grade 2	0 (0)
	Grade 3	13 (52)
	Grade 4	3 (12)
	Grade 5	0 (0)

The definitions of severity grade were based on anaphylaxis guidelines

Table E8a – Diagnostic performance of specific IgE measurement to fish allergen extracts and components in **grass carp-challenge** subjects

	Cod extract	Salmon extract	Cod PV (rGad c 1)	Common Carp PV (rCyp c 1)	SPT fish mix	SPT salmon
AUC (95% CI)	0.854 (0.709 - 1.000)	0.701 (0.503 - 0.900)	0.920 (0.813 - 1.027)	0.917 (0.810 - 1.024)	0.809 (0.631 - 0.987)	0.747 (0.561 - 0.932)
Sensitivity (%)	77.8	72.2	88.9	100.0	88.9	77.8
Specificity (%)	75.0	62.5	37.5	25.0	50.0	50.0
PPV (%)	87.5	81.3	76.2	75.0	80.0	77.8
NPV (%)	60.0	50.0	60.0	100.0	66.7	50.0
Optimal cut-off value* (>, kUA/L)	0.47	0.83	0.91	2.14	4	5

AUC, area under the curve; kUA/L, Kilounits of allergen-specific IgE per litre; PPV, positive predictive value; NPV, negative predictive value; PV, parvalbumin

*Optimal cut-off values were calculated based on calculation of the Youden's indices

Table E8b – Diagnostic performance of specific IgE measurement to fish allergen extracts and components in **salmon-challenge** subjects

	Cod extract	Salmon extract	Cod PV (rGad c 1)	Common Carp PV (rCyp c 1)	SPT fish mix	SPT salmon
AUC (95% CI)	0.746 (0.507 - 0.985)	0.881 (0.733 - 1.029)	0.659 (0.382 - 0.936)	0.603 (0.298 - 0.909)	0.564 (0.320 - 0.808)	0.659 (0.435 - 0.883)
Sensitivity (%)	83.3	100.0	83.3	83.3	83.3	100.0
Specificity (%)	42.9	47.6	23.8	9.5	19.1	38.1
PPV (%)	29.4	35.3	23.8	20.8	22.7	31.6
NPV (%)	90.0	100.0	83.3	66.7	80.0	100.0
Optimal cut-off value* (>, kUA/L)	1.15	0.63	5.14	8.7	4	2

AUC, area under the curve; kUA/L, Kilounits of allergen-specific IgE per litre; PPV, positive predictive value; NPV, negative predictive value; PV, parvalbumin

*Optimal cut-off values were calculated based on calculation of the Youden's indices