Maternal consumption of seafood in pregnancy and child neuropsychological development: A longitudinal study based on a population with high consumption levels

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ABSTRACT

Seafood consumption during pregnancy is thought to be beneficial for child neuropsychological development, but no large cohort studies with high fatty fish consumption have analyzed the association by seafood subtype. 1,892 and 1,589 mother-child pairs were assessed at the ages of 14 months and 5 years respectively, in a population-based Spanish birth cohort established during 2004-2008. Bayley and McCarthy scales and Childhood Asperger Syndrome Test were used for neuropsychological development. Multivariate linear regression models were adjusted for socio-demographic characteristics and further adjusted for cord blood mercury or long chain polyunsaturated fatty acid concentrations. Overall, consumption of seafood above the recommended limit of 340 g/week was associated with increments in neuropsychological scores. By subtypes, in addition to lean fish, large fatty fish consumption showed a positive association (those within the highest quantile (>238 g/week) had an adjusted increase of +2.29 points in the McCarthy general cognitive scale (95% confidence interval [CI] 0.42, 4.16)). Similar findings were observed in Childhood Asperger Syndrome Test. Coefficients diminished 15-30 % after adjusting for mercury or long chain polyunsaturated fatty acid concentrations. Consumption of large fatty fish during pregnancy presents moderate child neuropsychological benefits, including improvements in cognitive functioning and some protection from autistic spectrum traits.

Keywords

Seafood Intake; Pregnancy; Neuropsychological Development; Autistic Spectrum Traits; Fatty Acids; Mercury; Population-based Birth Cohort.

Abbreviations

DHA (Docosahexanoic Acid); β Coeff (Beta Coefficient); CI (Confidence Interval); CAST (Childhood Asperger Syndrome Test); DDE (2,2-bis(p-chlorophenyl)-1,1-dichloroethylene); FFQ (Food Frequency Questionnaire); Hg (Mercury); LCPUFAs (Long Chain Poly-unsaturated Fatty Acids); MSCA (McCarthy Scales of Children's Abilities); PCBs (Polychlorinated Biphenyls); PC50 (50^{th} Percentile).

INTRODUCTION

Maternal consumption of seafood during pregnancy has been associated with improvements in neuropsychological development among children in several studies (1, 2). These beneficial associations are thought to be at least partly attributable to higher intakes of key nutrients, including the long chain poly-unsaturated fatty acids (LCPUFAs) such as omega-3 docosahexanoic acid (DHA) which is essential for optimal prenatal neurodevelopment (3), particularly during early stages of brain formation (3, 4). DHA is not widely distributed in the diet, and seafood, particularly fatty species, are the major source (1, 3).

Nonetheless, elevated intakes of seafood during pregnancy are of concern, as seafood is an important source of neurotoxic contaminants such as methylmercury and organochlorine compounds, which have been associated with decrements in child neuropsychological scores (1, 2). To help balance these risks and benefits, guidelines issued in the USA, UK and EU advise pregnant women regarding seafood intakes (1, 5, 6). The US Food and Drug Agency 2014 draft recommendations (6) emphasize selecting subtypes of seafood lower in these contaminants, with consistent advice to avoid large predatory fish such as shark, swordfish, king mackerel and tilefish, and to limit consumption of albacore tuna, despite, large fatty fish such as tuna containing some of the highest levels of DHA (7).

These guidelines have been debated, as some studies found no evidence of adverse associations with maternal seafood consumption exceeding 340 g per week, the current recommended limit in the USA. However, the recently issued scientific opinion report of the European Food Safety Authority concluded with a less restricted recommendation, in which beneficial health associations are limited to 1-4 fish servings per week (150-600 g), despite the uncertainties of serving sizes in the European epidemiological studies (5, 8, 9). The studies supporting the guideline statements did not, however, examine associations with different subtypes of seafood (2). Thus at present, there is insufficient knowledge on the association between seafood consumption in pregnancy and child neuropsychological outcomes.

The present study examines maternal seafood consumption in pregnancy and child neuropsychological development in a Spanish multicentre cohort, where average consumption exceeds current US recommended levels, and allows the study of numerous seafood subtypes. We aimed to assess associations of consumption of large and small fatty fish, lean fish and shellfish as well as total seafood with a range of neuropsychological outcomes including cognitive and motor functioning, and autistic spectrum traits, at two ages (14 months and 5 years). We also investigated the roles of mercury (Hg) and LCPUFA in cord blood, maternal biomarkers of other environmental pollutants and nutrition, and child seafood consumption on these associations.

METHODS

Subjects

The Spanish Environment and Childhood (Infancia y Medio Ambiente, INMA) cohorts included in this analysis were established between 2004 and 2008 in the 4 regions of Asturias, Gipuzkoa (the Basque Country), Sabadell (Catalonia) and Valencia (10). Participant recruitment and follow-up procedures have been reported in detail elsewhere (10). A total of 2,644 eligible women were recruited during prenatal visits in the first trimester of pregnancy. Women agreed to participate and met the inclusion (≥16 years of age, singleton pregnancy, intention to deliver at the reference hospital) and exclusion (communication handicap, fetuses with malformations, assisted conception) criteria. Women were followed-up during pregnancy and their children enrolled at birth and followed up until age 5 years. After excluding women who withdrew, were lost to follow-up, or underwent abortions or fetal deaths, a total of 2,506 pregnant women were monitored through delivery. Final analyses included 1,892 children at 14 months and 1,589 children at 5 years old. The analysis excluded 93 preterm infants (<37 weeks gestation) known to differ from term births with respect to neuropsychological development (11), and 18 children with pathologies including plagiocephaly. 522 children were lost to follow up at age of 14 months and 341 were lost to follow up at age of 5 years. The remaining missing cases are attributable to missing data on some covariates. All participants provided written informed consent, and the study was approved by hospital and institutional ethics committees in each region. Further information is shown in Web Appendix 1.

Exposure and covariate information

Questionnaires, completed twice during pregnancy and at child ages of 14 months and 5 years, were administered by trained interviewers to obtain extensive information on maternal and child characteristics (Web Appendix 1, Web Tables 1-3).

We used a semi-quantitative Food Frequency Questionnaire (FFQ) of 101 food items to assess the usual daily intake of foods and nutrients at 10-13 weeks of pregnancy and again at 28-32 weeks. The FFQ was a modified version of a previous FFQ based on the Harvard questionnaire (12), adapted and validated among the pregnant women of the Valencia cohort (13). Further information is provided in Web Appendix 1. Women reported their usual intake of foods from the last menstruation to the first prenatal visit, using reference portions and nine frequency categories ranging from never/less than once a month to more than six times per day. The questionnaire included 10 seafood items. The response to each seafood item was converted to average weekly intakes in grams; then summed to compute the total and seafood subtypes (in g/week). Seafood was classified *a priori* as follows: (i) large fatty fish, including one item from the questionnaire ('baked or steamed larger fatty fish such as tuna, swordfish, albacore'); (ii) smaller fatty fish species, including two items from the questionnaire ('baked or steamed smaller fatty fish such as mackerel, sardines, anchovies, salmon'; 'tinned sardines/mackerel'); (iii) lean fish, including three items from the questionnaire ('fried fish'; 'baked or steamed lean fish such as hake, sole, or bream'; 'tinned tuna' which has similar levels of DHA and Hg as lean fish); (iv) shellfish, including three items from the questionnaire ('shrimp, prawns, lobster or crab'; 'clams, mussels, oysters'; 'squid, octopus, cuttlefish'); (v) smoked/ salted fish, including one item from the questionnaire ('salted or smoked fish: anchovies, cod, salmon'); and (vi) overall seafood intakes, calculated as the sum of consumption of all items. The (v) subtype group was excluded from individual analyses due to its low frequency in this cohort. Intakes were adjusted for energy intake using the residual method (14); and analyzed primarily in quantile categories of weekly grams. The quantile categories were created prior to analysis. For some of the seafood subtypes, the number of quantiles created was constrained by low intake frequency. In order to check potential systematic bias in reporting by education level, we checked whether the intake sum of means by seafood subtype was similar to the mean of total intake per each education level category, resulting in no mean differences (data not shown). Similar methods were applied to estimate seafood consumption of mothers at 28-32 weeks of pregnancy and seafood consumption of children at age of 5 years.

Dietary intakes of omega-3 were estimated from the FFQ and use of supplements of omega-3, iodine and folic acid were recorded (13). Cord blood mercury and total fatty acid levels (including DHA), maternal organochlorine serum levels, and, plasma 25(OH)D_3 and urinary iodine concentrations during pregnancy were measured as previously described (extended information in Web Appendix 1 and Web Tables 1-3).

Neuropsychological assessments

Child neuropsychological development was assessed at ages of 14 months and 5 years using the Bayley Scales of Infant Development (15) and the McCarthy Scales of Children's Abilities (MSCA) (16), respectively. Five-year-old autistic spectrum traits were assessed using the Childhood Asperger Syndrome Test (CAST) (17), which was administered after the MSCA test session. Further information is in Web Appendix 1 and Web Table 3.

Statistical analysis

Associations between total and different subtypes of seafood consumption and neuropsychological scores were evaluated using separate multivariate linear regression analyses. Seafood consumption was evaluated both as ordinal (quantiles) and continuous (10 g/week) variables (after testing linearity by generalized additive models (GAM)). Tests for linear trend were performed by including median values of consumption within each quantile category in the regression models. Minimally-adjusted regression models included age and gender of the child, cohort, total energy intake (kcal), and quality of the test (good, regular or low) recorded by the psychologist after examination. Other important variables, evaluated as potential confounders and mediators, are described in Web Tables 1-3. Confounders were retained only if they modified the coefficient of the seafood consumption parameter in the basic model by >5% (18). The final models further adjusted for child gestational age and weight at birth, duration of breastfeeding, maternal age, education level, social class, parity, pre-pregnancy BMI and country of origin/birth.

Sensitivity analyses adjusted for concentrations of cord blood Hg and LCPUFA and other biomarkers and food supplements (see them listed in Web Tables 1-3), through inclusion in regression models as continuous variables. Generalized additive models were used to assess the linearity assumption. As a result, the Hg variable was $\log_{10}$ transformed to achieve linearity. In secondary analyses, the final models were repeated with maternal seafood consumption during the third trimester of pregnancy (Spearman r=0.50, first-trimester-consumption) and child seafood consumption (r=0.22, first-trimester-consumption) as independent variables; excluding tinned tuna from lean fish subtype; changing the reference group of total seafood consumption to ≤ 340 g per week; and stratifying analyses by geographical location, Cantabric sea (Asturias + Gipuzkoa) vs. Mediterranean sea (Sabadell + Valencia). All analyses were conducted with the STATA 12 statistical software package.

RESULTS

The reported average of total seafood consumption was 498 grams per week (a median of 454 g/week), which is considered to be about three servings per week in the European Food Safety Authority. Very few (n=15, 0.8 %) were non-consumers. As shown in Table 1, overall seafood consumption during early pregnancy was generally related to maternal and child characteristics. Intakes were higher among mothers who were older, born in Spain, had higher socioeconomic and education status, did not smoke during pregnancy, but used alcohol, breastfed for longer periods, and had higher cord blood mercury and LCPUFA levels. For specific subtypes of seafood, consumers showed similar characteristics. The Spearman correlation coefficients between intakes of different seafood subtypes were positive, albeit weak to moderate. Among them, the strongest correlation was r=0.29 between large fatty fish and lean fish. Large fatty fish consumption showed the strongest Spearman correlation coefficient with Hg cord blood levels (r=0.34) and DHA levels (r=0.20).

Total seafood consumption associations with the main neuropsychological outcomes are shown in Figure 1, with the strongest associations observed in the outcome scores (MSCA and CAST) measured at 5-years. In Figure 2, McCarthy sub-area scales are presented; positive associations were observed among all scales with the largest coefficients generally found in seafood quantile 4 (median=600 g/week or about 4 servings/week).

Minimally and fully adjusted associations between maternal seafood subtype consumption and Bayley mental scale at 14 months of age are shown in Table 2. Positive associations were observed for lean fish and small fatty fish, the latter with a trend (*p* for trend=0.03). Associations with the Bayley psychomotor scale were somewhat weaker (Web Table 4). A positive trend in MSCA general cognitive score was found with large fatty fish intake (*p* for trend= 0.020), and a weak trend with lean fish (*p* for trend= 0.110) (Table 3). Generally, when using categorical variables of seafood consumption in the regression models, quantiles 3 and 4 tended to show the largest coefficients and a slight decrease in the last quantile (Table 2 and 3).

As shown in Table 4, maternal seafood consumption, total and by subtype, was generally associated with a reduction of number of traits of CAST. Lean fish intake showed an association with the outcome from quantile 2. A trend (*p* for trend=0.013) was observed for large fatty fish intake. In all models presented in Tables 2, 3 and 4, shellfish intake presented the weakest associations.

Similar results were observed after excluding tinned tuna from lean fish subtype and when tinned tuna was treated as an independent variable (data not shown), and when the reference group of total seafood consumption included all mothers with intakes ≤ 340 g per week (Web

Table 5). Large fatty fish association with MSCA general score was similar after adjustment for lean fish intake (Q4 versus Q1, β = 2.00, 95% CI=0.07 to 5.60, *p* for trend=0.047). Associations were somewhat weaker when seafood consumption was assessed in the third trimester of pregnancy (Web Table 6). Association patterns were similar when the data was stratified by Cantabric and Mediterranean Sea locations (Web Table 7). The inclusion of the 93 preterm children or the exclusion of large fatty fish eaters (n=886) did not change results (data not shown).

The associations with the MSCA scales (Table 3) were attenuated by 15-30 % after further adjustment for Hg and LCPUFA cord blood levels in separate models (Web Table 8). Separate models adjusting for organochlorines (polychlorinated biphenyls (PCBs) and 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE)), iodine and 25(OH)D3 levels, LCPUFA intake estimations, and supplements of LCPUFA, folic acid and iodine during pregnancy did not affect the results (data not shown).

Current child seafood consumption and MSCA general cognitive score showed similar but weaker association patterns than that described in Table 3 with maternal seafood consumption. After adjustment for maternal consumption, the coefficients of the models reduced by 21 % (Web Table 9). Maternal consumption association was similar to previous models (Table 3) after adjustment for child consumption (data not shown).

DISCUSSION

This study, conducted in a population characterized by high seafood consumption, found moderate positive associations between seafood consumption during pregnancy and child neuropsychological development, particularly at five years of age. Small fatty fish intake explained part of the positive associations at 14 months old and lean and large fatty fish appeared to be child neuropsychological predictors at five years. As a new finding, a consistent reduction of autistic spectrum traits was also observed with total, lean and large fatty fish consumption. These associations generally remained positive above the recommended USA current guidelines (340 g total fish consumption per week during pregnancy) (5). Only part of these associations was reduced by adjustments for Hg and LCPUFA cord blood levels. Child seafood consumption showed similar results with somewhat reduced associations after controlling for maternal consumption.

Average seafood consumption in this population (498 g per week, about 3 servings per week or 71g/day), was similar to levels reported in other Spanish studies (e.g. 72g/day reported in the Basque Country) (19). These high consumption levels facilitated the analyses of associations of intakes substantially exceeding recommended levels, as well as of specific seafood subtypes, with the outcomes of interests. Thus unlike previous studies, we were able to examine how maternal consumption of subtypes of seafood specified in the recommendations—most notably larger versus smaller fatty fish species—related to child neuropsychological development. The positive association observed for MSCA cognitive scores among those consuming moderate amounts of large fatty fish would suggest that some guidelines may be slightly stringent (6). In fact, the present findings tend to support recent recommendations issued by the European Food Safety Authority, which are less restrictive in limiting seafood consumption and concluded that there are no adverse health associations when exceeding the amount recommended for positive health associations, which is 1-4 fish servings per week (150-600 g) (9). Furthermore, given that the associations observed using seafood consumption in pregnancy were stronger than using seafood intake in childhood as exposure, uterine life seems to be an important window for neurodevelopment, particularly during the early pregnancy period where there is intense activity in neuron formation, differentiation and migration (4).

We identified some studies evaluating the association of prenatal seafood intake and neuropsychological development (2). Most studies reported positive associations with a wide range of outcomes, such as, neurological development, motor development, verbal intelligence quotient, perception, social behavior and less inattention and hyperactivity (2, 8, 20, 21). In just a few of them, there was an attenuation of positive association in the highest seafood intake category (2, 21). Our findings support the idea of a general beneficial association of brain development and a potential light attenuation at the highest levels of consumption. The surprising protective association with spectrum autistic traits has not been previously reported, although pro-social behavior improvements were observed in a previous study (8) and children with autistic spectrum traits tend to show lower pro-social behaviors (22). One potential pathway could be through LCPUFA, particularly DHA intake from seafood (23). Several LCPUFA controlled trials and observational studies have reported improvements in cognition, ADHD and antisocial symptoms (24). Our findings showing a moderate attenuation after adjusting for LCPUFA (including DHA) levels in cord blood are supportive of that hypothesis, given that other potential intermediate factors, such as vitamin D and iodine levels, in pregnancy did not explain any of the observed associations.

While a few previous studies on seafood consumption during pregnancy and child neuropsychological development have examined seafood subtypes (2, 25), none (to our knowledge) have separately examined associations with large versus small species of fatty fish. Somewhat inconsistently with our findings, one previous study reported that while fatty fish of any kind consumed less than once per week was associated with small increases in intelligence quotient at age nine, no associations were observed at higher levels of intake (21).

Current guidelines for seafood consumption during pregnancy have been developed largely based on evidence linking mercury and other contaminants frequently found in marine foods with poorer neuropsychological development (1). Large fatty fish are of particular concern, as these long lived, predator species may accumulate both mercury and lipophilic contaminants such as organochlorines (26, 27). In the few previous studies where such measurements were available, positive associations between seafood consumption and child neuropsychological development were strengthened or not influenced by adjustments for cord blood Hg, PCBs or DDE (2, 26). In this study, there was an attenuation of the association estimate after adjusting of cord blood levels of Hg. In this regard the precision of the independent variables (both the toxicant exposure and the beneficial dietary factors and other confounders) in our study are of importance. If the toxicant is measured with a greater precision than the dietary factor through FFQ, the association of the later will generally be biased toward the null (28). Probably, in the present study, cord blood mercury was an indicator of seafood consumption.

The complexity of separating positive and adverse associations of seafood consumption and methylmercury (or Hg), respectively, on child neuropsychological development has been discussed elsewhere (29). Several factors may be masking any adverse association with methylmercury intake. For example, pregnant women with higher socioeconomic status tend to consume more seafood and be exposed to higher levels of methylmercury, but their children tend to perform better on cognitive tests (28). Additionally, variability in levels of both methylmercury and DHA are dependent on seafood subtype, with larger predators containing higher levels of methylmercury, but some, such as tuna fish, also containing higher concentrations of DHA (7). These factors and potential genetic vulnerabilities to methylmercury toxicity (28), make objective evaluation of the toxic risk of this exposure difficult, particularly since such exposure is closely linked to total seafood intake, which confers benefits on neuropsychological development.

A two point increase in child's cognitive score is not remarkable for an individual but important for the population. If a specific population, particularly a community with poor seafood consumption, benefited from greater consumption of seafood, the Gaussian distribution for scores would likely shift to the right. As an end result, the chance of finding 'borderline' children will be diminished. If these beneficial associations are permanent, they could be related to social and economic positive changes (18, 30). Although we experienced a moderate loss to follow up of 40 percent, this allows some generalizability of our findings.

Although this study contained more information on seafood subtypes than earlier papers, we observed moderate correlations between them limiting the interpretation of fully independent associations by subtypes. Moreover, while FFQ are valid tools to assess dietary intakes, the use of self-reported data is a major limitation in this field of research due to an increased level of noise related to the subjectivity in recall of food habits and the potential influence of socio-cultural background. Additionally, healthy nutritional habits that include more seafood consumption are also related to higher maternal intelligence quotient and education level, and lower smoking habits during pregnancy (2); hence, we cannot rule out some residual confounding. However, we have carefully considered a wide range of potential confounders, including the ones mentioned above (Web Tables 1-3), and sensitivity analyses to address this potential limitation. Negative confounding by Hg exposure was not found here, probably due to the lack of an observed negative association of Hg, as reported previously in this cohort

(31). The difficulty of disentangling both associations demonstrates the statistical limitations of epidemiological studies. Finally, we found moderate association trends in some of the seafood subtypes; but there was also a weak tendency of saturation in the highest quantiles of the exposure, probably the association pattern is not completely linear, with stronger positive associations in the moderately high seafood consumption categories.

Overall, the present results suggest no adverse associations of high seafood consumption in pregnancy on offspring neurodevelopment. Moderate consumption of small and large fatty fish and lean fish in pregnancy are associated with moderate improvements in child neuropsychological development, including cognitive functions and autistic spectrum traits. A slight dilution of the association at the highest intake levels may be indicative of a weak counter-balancing association due to the potential harm of related contaminants. The moderate mediation role of LCPUFAs observed here suggests they may have a mechanistic role. The role of Hg was difficult to disentangle since it appeared to be a stronger biomarker of seafood consumption rather than having any expected neurotoxic association. Finally, the present findings should be taken with caution and future research should focus on older children in order to further explore whether the association patterns observed here continue into later life, with particular attention given to large fatty fish species.

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Table 1. Maternal pregnancy first trimester seafood consumption by socio-demographic and environmental characteristics[b] (INMA Study, 2004-2008)

		Seafood Intake, g/week				
	N	Total (50th Percentile)	Large Fatty Fish (50th Percentile)	Smaller Fatty Fish (50th Percentile)	Lean Fish (50th Percentile)	Shellfish (50th Percentile)
Maternal characteristics						
Age						
<31 y	946	422[a]	0[a]	14[a]	260[a]	45[a]
>=31 y	1049	482[a]	50[a]	31[a]	296[a]	52[a]
Education						
≤Primary	424	401[a]	0[a]	20	228[a]	44[a]
Secondary	829	460[a]	42[a]	25	287[a]	52[a]
University	738	473[a]	49[a]	29	304[a]	49[a]
Social class						
High Skilled	815	483[a]	50[a]	35[a]	308[a]	53[a]
Non-manual	730	439[a]	38[a]	0[a]	268[a]	47[a]
Manual	423	434[a]	0[a]	28[a]	352[a]	46[a]
Pre-pregnancy BMI						
Tertile 1	647	439	31[a]	26	274[a]	47
Tertile 2	675	465	47[a]	23	296[a]	50
Tertile 3	673	459	46[a]	27	274[a]	51
Parity						
Nulliparous	1135	457	44	20[a]	282	51[a]
Parous	858	453	41	31[a]	284	47[a]
Born in Spain						
Yes	1858	463[a]	45[a]	28[a]	288[a]	50[a]
No, Latin America	88	322[a]	0[a]	0[a]	171[a]	33[a]
No, other place	46	421[a]	0[a]	0[a]	263[a]	30[a]
Smoked entire pregnancy						
No	1630	462[a]	45[a]	29[a]	288[a]	49[a]
Yes	333	423[a]	13[a]	18[a]	248[a]	46[a]
Alcohol use entire pregnancy						
No	1040	436[a]	37[a]	0[a]	275[a]	45[a]
Yes	929	475[a]	47[a]	36[a]	290[a]	52[a]
Child characteristics						
Gender						
Male	1016	452	41	31[a]	280	48
Female	979	454	44	20[a]	284	50
Birthweight						
<3000 g	458	439	46	0[a]	279	48
3000-3500 g	939	459	41	35[a]	284	51
>3500 g	585	451	44	15[a]	283	47
Gestational age						
≤40 weeks	1123	465[a]	46[a]	24	291[a]	47
>40 weeks	872	444[a]	38[a]	26	270[a]	51

Breastfeeding duration (any)						
<=24 weeks	1078	439[a]	40	0[a]	278[a]	48
>24 weeks	872	475[a]	45	36[a]	286[a]	50
Cord blood mercury levels (mcg/l)						
< 8.5	746	396[a]	0[a]	24	248[a]	44[a]
≥ 8.5	795	509[a]	60[a]	30	325[a]	54[a]
Omega-6 / Omega-3 ratio in cord blood levels						
<=PC50	389	511[a]	49[a]	46[a]	324[a]	54[a]
>PC50	379	423[a]	0[a]	22[a]	259[a]	43[a]

[a]P-value<0.10 of differences between groups per seafood subtype using [b]Wilcoxon *rank-sum test*. PC50=50[th] Percentile. Y=Year.

Table 2. Associations between maternal pregnancy first trimester seafood consumption and child Bayley Mental Scale at 14 months (INMA Study, 2004-2008)

Seafood intake (g/w)[f]	N subjects	Minimally adjusted[a]		Fully adjusted[b]	
		β Coeff	95% CI	β Coeff	95% CI
Total seafood consumption (10g/w)[g]	1892	0.02[d]	-0.00, 0.06	0.02[d]	-0.00, 0.05
Quantiles: 1[c]	383	0.00	Referent	0.00	Referent
2	392	2.13[d]	-0.03, 4.29	2.14[e]	0.00, 4.28
3	364	1.53	-0.67, 3.73	1.28	-0.91, 3.47
4	386	3.03[e]	0.84, 5.22	2.90[e]	0.72, 5.09
5	367	2.02[d]	-0.19, 4.22	2.06[d]	-0.13, 4.26
p-for-trend		*0.08*		*0.08*	
Large fatty fish (10g/w)[g]	1892	0.00	-0.07, 0.08	0.00	-0.07, 0.08
Quantiles: 1	853	0.00	Referent	0.00	Referent
2	341	0.28	-1.66, 2.22	0.08	-1.84, 2.01
3	345	-0.16	-2.12, 1.79	-0.12	-2.06, 1.82
4	353	0.71	-1.24, 2.67	0.51	-1.43, 2.46
p-for-trend		*0.510*		*0.623*	
Small fatty fish (10g/w)[g]	1892	0.06	-0.02, 0.14	0.06	-0.02, 0.15
Quantiles: 1	877	0.00	Referent	0.00	Referent
2	333	2.16[e]	0.14, 4.19	1.79[d]	-0.22, 3.80
3	338	-0.47	-2.40, 1.46	-0.37	-2.29, 1.55
4	344	2.63[e]	0.71, 4.55	2.45[e]	0.54, 4.36
p-for-trend		*0.02*		*0.03*	
Lean fish (10g/w)[g]	1892	0.03[d]	-0.00, 0.07	0.03[d]	-0.00, 0.07
Quantiles: 1	387	0.00	Referent	0.00	Referent
2	386	0.93	-1.23, 3.10	0.44	-1.71, 2.58
3	380	2.35[e]	0.14, 4.56	2.07[d]	-0.12, 4.26
4	372	1.55	-0.66, 3.75	1.41	-0.78, 3.59
5	367	2.00[d]	-0.24, 4.24	1.77	-0.46, 3.99
p-for-trend		0.09		0.10	
Shellfish (10g/w)[g]	1892	0.04	-0.07, 0.16	0.05	-0.06, 0.16
Quantiles: 1	373	0.00	Referent	0.00	Referent
2	370	1.10	-1.13, 3.34	0.80	-1.41, 3.01
3	384	2.26[e]	0.07, 4.44	1.86[d]	-0.30, 4.03
4	394	1.65	-0.53, 3.83	1.45	-0.70, 3.61
5	371	1.71	-0.55, 3.97	1.52	-0.72, 3.75
p-for-trend		*0.18*		*0.21*	

[a]Regression models adjusted for: gender of the child, age during testing, cohort, quality of the test and maternal energy (Kcal) intake during pregnancy. [b]Regression models additionally adjusted for: child birth weight, gestational age and duration of breastfeeding, maternal age, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [c]Results were similar when reference group included all mothers eating ≤ 340 g per week (Web Table 5). [d]P-value<0.10. [e]P-value<0.05. [f]Median of total seafood intake per each Quantile (Q), g per week: Q1=195g; Q2=338g; Q3=461g; Q4=600g; Q5=854g. Median of large fatty fish intake: Q1=None; Q2=48g; Q3=92g; Q4=238g. Median of small fatty fish intake: Q1=None; Q2=37g; Q3=69g; Q4=147g. Median of lean fish intake: Q1=90g; Q2=192g; Q3=286g; Q4=382g; Q5=557g. Median of shellfish intake: Q1=None; Q2=27g; Q3=49g; Q4=76g; Q5=139g. [g]10g/week increase. CI=Confidence Interval. W=Week.

Table 3. Associations between maternal pregnancy first trimester seafood consumption and child McCarthy General Cognitive Scale at 5 years (INMA Study, 2004-2008)

Seafood intake (g/w)[f]	N subjects	Minimally adjusted[a]		Fully adjusted[b]	
		β Coeff	95% CI	β Coeff	95% CI
Total seafood consumption (10g/w)[g]	1589	0.03[e]	0.00, 0.05	0.02[d]	0.00, 0.05
Quantiles: 1[c]	320	0.00	Referent	0.00	Referent
2	340	1.91[d]	-0.23, 4.04	1.61	-0.43, 3.65
3	299	3.46[e]	1.24, 5.67	2.13[e]	0.00, 4.26
4	323	3.60[e]	1.41, 5.79	2.84[e]	0.74, 4.94
5	308	2.93[e]	0.72, 5.14	2.08[d]	-0.04, 4.21
p-for-trend		*0.007*		*0.049*	
Large fatty fish (10g/w)[g]	1589	0.10[e]	0.02, 0.17	0.06[d]	-0.00, 0.13
Quantiles: 1	704	0.00	Referent	0.00	Referent
2	285	2.99[e]	1.05, 4.93	2.26[e]	0.40, 4.11
3	296	2.36[e]	0.43, 4.30	1.93[e]	0.09, 3.79
4	304	3.46[e]	1.51, 5.40	2.29[e]	0.42, 4.16
p-for-trend		*0.001*		*0.02*	
Small fatty fish (10g/w)[g]	1589	-0.03	-0.11, 0.05	-0.03	-0.10, 0.05
Quantiles: 1	736	0.00	Referent	0.00	Referent
2	280	1.41	-0.61, 3.44	0.60	-1.33, 2.53
3	288	0.94	-0.98, 2.87	1.25	-0.59, 3.10
4	285	1.27	-0.67, 3.21	0.91	-0.93, 2.76
p-for-trend		*0.18*		*0.25*	
Lean fish (10g/w)[g]	1589	0.04[e]	0.00, 0.08	0.03	-0.01, 0.06
Quantiles: 1	328	0.00	Referent	0.00	Referent
2	325	2.65[e]	0.49, 4.80	1.76[d]	-0.29, 3.81
3	322	2.79[e]	0.60, 4.99	2.01[d]	-0.08, 4.11
4	307	3.42[e]	1.20, 5.62	2.47[e]	0.36, 458
5	307	3.01[e]	0.78, 5.25	1.89[d]	-0.25, 4.03
p-for-trend		*0.017*		*0.11*	
Shellfish (10g/w)[g]	1589	-0.03	-0.15, 0.09	-0.02	-0.13, 0.09
Quantiles: 1	307	0.00	Referent	0.00	Referent
2	307	0.09	-2.16, 2.35	-0.12	-2.26, 2.02
3	331	1.16	-1.02, 3.35	0.81	-1.27, 2.90
4	332	1.10	-1.09, 3.28	0.79	-2.29, 2.88
5	312	-0.83	-3.10, 1.44	-0.94	-3.10, 1.22
p-for-trend		*0.51*		*0.44*	

[a]Regression models adjusted for: gender of the child, age during testing, cohort, quality of the test and maternal energy (Kcal) intake during pregnancy. [b]Regression models additionally adjusted for: child birth weight, gestational age and duration of breastfeeding, maternal age, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [c]Results were similar when reference group included all mothers eating ≤ 340 g per week (Web Table 5). Further inclusion of all seafood subtypes in the final model showed similar association patterns (data not shown). [d]P-value<0.10. [e]P-value<0.05. [f]Median of seafood intake per each Quantile, g per week is shown in Table 2 footnotes. [g]10g/week increase. CI=Confidence Interval. W=Week.

Table 4. Associations between maternal pregnancy first trimester seafood consumption and Childhood Asperger Syndrome Test (CAST) at 5 years (INMA Study, 2004-2008)

Seafood intake (g/w)[f]	N subjects	Minimally adjusted[a]		Fully adjusted[b]	
		β Coeff	95% CI	β Coeff	95% CI
Total seafood consumption (10g/w)[g]	1393	-0.01[e]	-0.01, -0.00	-0.01[e]	-0.01, -0.00
Quantiles: 1[c]	289	0.00	Referent	0.00	Referent
2	294	-0.47[d]	-0.96, 0.03	-0.42[d]	-0.90, 0.07
3	271	-0.69[e]	-1.20, -0.18	-0.45[d]	-0.95, 0.05
4	280	-0.75[e]	-1.26, -0.24	-0.61[e]	-1.12, -0.11
5	260	-0.72[e]	-1.23, -0.20	-0.55[e]	-1.06, -0.04
p-for-trend		*0.006*		*0.037*	
Large fatty fish (10g/w)[g]	1393	-0.02[e]	-0.04, -0.01	-0.02[e]	-0.04, -0.00
Quantiles: 1	613	0.00	Referent	0.00	Referent
2	237	-0.42[d]	-0.88, 0.04	-0.32	-0.77, 0.13
3	269	-0.34	-0.79, 0.11	-0.28	-0.72, 0.16
4	274	-0.74[e]	-1.19, -0.29	-0.57[e]	-1.01, -0.13
p-for-trend		*0.002*		*0.013*	
Small fatty fish (10g/w)[g]	1393	-0.00	-0.02, 0.02	-0.00	-0.02, 0.01
Quantiles: 1	668	0.00	Referent	0.00	Referent
2	235	-0.36	-0.83, 0.12	-0.19	-0.66, 0.27
3	240	-0.15	-0.61, 0.30	-0.14	-0.59, 0.31
4	250	-0.45[e]	-0.90, 0.00	-0.37	-0.81, 0.07
p-for-trend		*0.056*		*0.11*	
Lean fish (10g/w)[g]	1393	-0.01[e]	-0.02, -0.00	-0.01[d]	-0.02, 0.00
Quantiles: 1	298	0.00	Referent	0.00	Referent
2	291	-1.03[e]	-1.52, -0.54	-0.89[e]	-1.37, -0.41
3	282	-0.91[e]	-1.41, -0.40	-0.77[e]	-1.26, -0.28
4	261	-0.63[e]	-1.14, -0.12	-0.48[d]	-0.98, 0.02
5	261	-0.92[e]	-1.45, -0.41	-0.70 [e]	-1.22, -0.19
p-for-trend		*0.017*		*0.10*	
Shellfish (10g/w)[g]	1393	0.02	-0.01, 0.04	0.01	-0.01, 0.04
Quantiles: 1	278	0.00	Referent	0.00	Referent
2	268	-0.12	-0.64, 0.40	-0.15	-0.66, 0.36
3	288	-0.61[e]	-1.17, -0.11	-0.58[e]	-1.08, -0.09
4	289	-0.12	-0.63, 0.38	-0.12	-0.61, 0.38
5	270	-0.07	-0.60, 0.45	-0.05	-0.57, 0.46
p-for-trend		*0.97*		*0.92*	

[a]Regression models adjusted for: gender of the child, age during testing, cohort, quality of the test and maternal energy (Kcal) intake during pregnancy. [b]Regression models additionally adjusted for: child birth weight, gestational age and duration of breastfeeding, maternal age, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [c]Results were similar when reference group included all mothers eating ≤ 340 g per week (Web Table 5). [d]P-value<0.10. [e]P-value<0.05. [f]Median of seafood intake per each Quantile, g per week is shown in Table 2 footnotes. [g]10g/week increase. CI=Confidence Interval. W=Week.

Figure 1. Associations[a] between maternal pregnancy first trimester total seafood consumption (Quantiles (Q) of g/week) and General Scores of Bayley, MSCA and CAST scales (INMA Study, 2004-2008)

[a]Regression models adjusted for: gender of the child, cohort, quality of the test, child birth weight, gestational age, duration of breastfeeding, child age during testing, maternal age, energy intake (Kcal) during pregnancy, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. Median of total seafood intake per each Quantile (Q), g per week: Q1=195g; Q2=338g; Q3=461g; Q4=600g; Q5=854g. The bars are depicting [95% CI], and each category is in comparison with Q1 which is depicted by the vertical line.

Figure 2. Associations[a] between maternal pregnancy first trimester total seafood consumption (Quantiles (Q) of g/week) and MSCA subarea scales (INMA Study, 2004-2008)

[a]Regression models adjusted for: gender of the child, cohort, quality of the test, child birth weight, gestational age, duration of breastfeeding, child age during testing, maternal age, energy intake (Kcal) during pregnancy, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. Median of total seafood intake per each Quantile (Q), g per week: Q1=195g; Q2=338g; Q3=461g; Q4=600g; Q5=854g. The bars are depicting [95% CI], and each category is in comparison with Q1 which is depicted by the vertical line.

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WEB APPENDIX 1

METHODS

Recruitment and inclusion criteria

Pregnant mothers were first approached by midwives at their local health centre and were given leaflets with information on the Environment and Childhood (Infancia y Medio Ambiente, INMA) project. Recruitment was carried out when mothers came in for their first obstetric ultrasound. At this point, INMA personnel (trained nurses) verified the inclusion criteria and sought informed consent for participation in the study.

The reference hospitals were: Sabadell (Hospital de Sabadell – Corporació Parc Taulí or Hospital de Terrassa), Valencia (Hospital La Fe), Asturias (Hospital Universitario Central de Oviedo), and Gipuzkoa (Hospital de Cruces in Barakaldo).

Mothers were considered eligible for inclusion if they were residents in the cohort area, at least 16 years old, were carrying a singleton pregnancy and planned to give birth in the reference hospital. Mothers who had participated in an assisted fertility programme or with communication difficulties were excluded.

For the regional cohorts, the proportion of participants in INMA out of the women identified as eligible was 60% for Sabadell, 54% in Valencia, 45% in Asturias and 68% in Gipuzkoa. The reasons given by eligible mothers (Sabadell and Gipuzkoa) for non-participation were the following: 27.6% did not wish to participate, 30.3% said they did not have the time to participate, 9.3% were not interested and 32.7% could not be located for a baseline interview after being identified as eligible. For Sabadell participants had a higher educational level, for Gipuzkoa a higher proportion of working mothers participated, for Valencia there was also a higher proportion of older women and working mothers. There were no differences between participants and non-participants in Valencia.

Exposure and covariate information

Trained interviewers administered the FFQ at 10-13 weeks of pregnancy, to estimate food intakes. FFQ validity in the estimation of seafood consumption was further supported by moderate correlations between total seafood intake and large fatty fish intake with cord blood LCPUFA levels (Arachidonic Acid (AA) / Eicosapentaenoic Acid (EPA) + DHA ratio) in a subset of 878 women (r = -0.15 and r = -0.20 respectively); lower correlations were found in another similar study (1).

Questionnaires administered in the first and third trimesters were used to obtain information on maternal characteristics including pre-pregnancy body mass index (BMI, kg/m^2), marital status, age, education level and occupational social class (2), reproductive history, country of origin/birth, smoking history and environmental tobacco exposure throughout pregnancy. FFQ during first trimester of pregnancy estimated intakes of omega-3 and recorded supplements of omega-3, iodine and folic acid (3). Weight gain during pregnancy was obtained from clinic records. Length of gestation was estimated from the date of the last menstrual period and first trimester ultrasound measures, the latter used when measures differed by more than seven days. Gender of the child, birth weight, birth length and Apgar scores were recorded by trained midwives at delivery. Information on postnatal tobacco exposure, the duration of predominant breastfeeding, and main child caregivers were collected in postnatal interviews at the time of the neurodevelopment testing. At the 5-year follow-up, parental psychopathological symptoms were assessed using the Revised Symptom Checklist (SCL-90-R). This is a self-reported questionnaire widely used in both typical and distressed populations. Ninety items are classified into 9 domains and a general score: somatization, obsessive–compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism. A general score SCL-90-R alpha coefficient of 0.97 was obtained for mothers. Additionally, the similarities subtest of the Wechsler Adult Intelligence, Third Edition, was used as a proxy for maternal verbal IQ.

Whole blood samples were collected by using venipuncture of cord vessels before the placenta was delivered. Samples were processed, separated into aliquots of 1 mL, and then frozen to −80°C until analysis.

One aliquot was used to analyze total mercury by thermal decomposition, amalgamation, and atomic absorption spectrometry by using a single-purpose AMA-254 advanced mercury analyzer (LECO Corporation, St. Joseph, Michigan). The analytical procedure has been described elsewhere (4)

Maternal serum collected in the first trimester was used to measure orgochlorine levels of 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE) and polychlorinated biphenyl (PCB) congeners 138, 153, and 180 (5). Samples were analyzed using gas chromatography with electron capture detector (GC-ECD). Data on these chemicals were not yet available for Asturias cohort; level estimates were available for 87.2% of women in the other centers. PCBs and DDE measurements were adjusted for lipids (6).

Maternal plasma 25(OH)D_3 and urinary iodine concentrations (UIC) during pregnancy were quantified (7, 8). Total fatty acids were analyzed in a subsample of cord plasma (n=878) by fast-gas chromatography. Hundred microlitres of sample were saponified by adding 1 ml of sodium methylate (0.5% w/v) and heating to 100 °C for 15 min (9). Cord plasma levels of AA, DHA and EPA were measured in order to create a ratio (AA/DHA+EPA), which reflects the LCPUFA levels in a balanced methodology.

Neuropsychological assessments

Child neurodevelopment was assessed at 14 months and 5 years using the Bayley Scales of Infant Development (10) and the McCarthy Scales of Children's Abilities (MSCA) (11). Psychologists flagged children whose tests were of poor quality due to lack of cooperation of the child (fatigue, bad mood, illness, etc): these factors were included in the models as a covariate. The Bayley Scales of Infant Development first Edition is one of the most psychometrically valid measurements for examining infants' mental and psychomotor development from 1 to 30 months of age. We used the mental scale (163 items) and the psychomotor scale (81 items). The MSCA includes six conventional scales: general cognitive, verbal, quantitative, memory, perceptive-performance, and motor functions. A new outcome scale for executive function was also created based on half of the MSCA subtests (12). Raw scores were centered to a mean of 100, with a standard deviation of 15 to compute index scores. This was to obtain indexes in accordance with a local normative sample and to avoid the use of US norms provided in the manual. Testing was conducted according to a strict protocol, including neuropsychologist training, and for a small number of children, multiple neuropsychologist evaluations were performed with results reached by consensus. Child autistic spectrum traits were assessed using the Childhood Asperger Syndrome Test (CAST) (13), which was administered after MSCA session. The rating-questionnaire is formed by 37 items that the evaluator administers to the child's parents. Each question can be scored with 0 or 1 points, except for 6 questions that do not score. The maximum possible score is 31 points. Furthermore, Cronbach’s Alpha Coefficient was used to determine the internal consistency per each of the scales and subscales. A good coefficient would be a value ≥ 0.70 (14): Scale Alpha Coefficients were > 0.70, except for MSCA motor subscale and CAST total score were of 0.64 each (good to moderate). Further information is published elsewhere (15, 16).

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WEB TABLE 1

Maternal pregnancy first trimester seafood consumption by sociodemographic and environmental characteristics,[b] extended list (INMA Study, 2004-2008)

		Seafood Intake, g/week				
	N	Total (50th Percentile)	Large Fatty Fish (50th Percentile)	Smaller Fatty Fish (50th Percentile)	Lean Fish (50th Percentile)	Shellfish (50th Percentile)
Maternal characteristics						
Age						
<31 y	946	422[a]	0[a]	14[a]	260[a]	45[a]
>=31 y	1049	482[a]	50[a]	31[a]	296[a]	52[a]
Education						
≤Primary	424	401[a]	0[a]	20	228[a]	44[a]
Secondary	829	460[a]	42[a]	25	287[a]	52[a]
University	738	473[a]	49[a]	29	304[a]	49[a]
Social class						
High Skilled	815	483[a]	50[a]	35[a]	308[a]	53[a]
Non-manual	730	439[a]	38[a]	0[a]	268[a]	47[a]
Manual	423	434[a]	0[a]	28[a]	352[a]	46[a]
Pre-pregnancy BMI						
Tertile 1	647	439	31[a]	26	274[a]	47
Tertile 2	675	465	47[a]	23	296[a]	50
Tertile 3	673	459	46[a]	27	274[a]	51
Proxy Verbal IQ						
>=8	1206	450	44	26	285	51
<8	382	445	39	20	266	47
Psychopath Symp.						
<65	1372	449	44	24	277	51[a]
>=65	128	412	35	21	250	43[a]
Parity						
Nulliparous	1135	457	44	20[a]	282	51[a]
Parous	858	453	41	31[a]	284	47[a]
Smoked entire pregnancy						
No	1630	462[a]	45[a]	29[a]	288[a]	49[a]
Yes	333	423[a]	13[a]	18[a]	248[a]	46[a]
Alcohol use entire pregnancy						
No	1040	436[a]	37[a]	0[a]	275[a]	45[a]
Yes	929	475[a]	47[a]	36[a]	290[a]	52[a]
Main child minder-14 months						
Mother	1107	452	40[a]	23	271	48
Both parents	364	474	50[a]	29	291	50
Other	448	456	40[a]	30	291	54
Born in Spain						
Yes	1858	463[a]	45[a]	28[a]	288[a]	50[a]
No, Latin America	88	322[a]	0[a]	0[a]	171[a]	33[a]
No, other place	46	421[a]	0[a]	0[a]	263[a]	30[a]

Omega-3 Supp. Preg.						
No	1790	456	42[a]	25	284	49
Yes	205	451	48[a]	22	271	48
Iodine Supp. Preg						
No	710	440	0[a]	31	276	53[a]
Yes	1285	461	48[a]	21	286	47[a]
Folate Supp. Preg						
No	130	440	38	42[a]	272	50
Yes	1782	455	43	25[a]	284	49
1st trim. serum PCBs(ng/g)[c]						
< 110.92	735	432[a]	0[a]	24[a]	268[a]	47
≥ 110.92	764	466[a]	46[a]	42[a]	286[a]	48
1st trim. serum DDE (ng/g)[c]						
< 123.15	752	468	38	31[a]	287	51[a]
≥ 123.15	747	439	13	40[a]	271	47[a]
Child characteristics						
Gender						
Male	1016	452	41	31[a]	280	48
Female	979	454	44	20[a]	284	50
Birthweight						
<3000 g	458	439	46	0[a]	279	48
3000-3500 g	939	459	41	35[a]	284	51
>3500 g	585	451	44	15[a]	283	47
Gestational age						
≤40 weeks	1123	465[a]	46[a]	24	291[a]	47
>40 weeks	872	444[a]	38[a]	26	270[a]	51
Breastfeeding duration (any)						
<=24 weeks	1078	439[a]	40	0[a]	278[a]	48
>24 weeks	872	475[a]	45	36[a]	286[a]	50
Cord blood mercury levels (mcg/l)						
< 8.5	746	396[a]	0[a]	24	248[a]	44[a]
≥ 8.5	795	509[a]	60[a]	30	325[a]	54[a]

[a]P-value <0.10. [b]Wilcoxon rank-sum test. [c]Sum of PCBs 118, 153, 138 and 180 (only Valencia, Guipúzkoa and Sabadell cohorts). Y=Year. DDE=2,2-bis(p-chlorophenyl)-1,1-dichloroethylene. PCBs=Polychlorinated Biphenyls.

WEB TABLE 2

Maternal pregnancy first trimester seafood consumption by potential intermediate factors,[b] extended list (INMA Study, 2004-2008)

	N	Seafood Intake, g/week Total (50th Percentile)	Large Fatty Fish (50th Percentile)	Smaller Fatty Fish (50th Percentile)	Lean Fish (50th Percentile)	Shellfish (50th Percentile)
Omega-6 /Omega-3 ratio in cord blood levels						
<=PC50	389	511[a]	49[a]	46[a]	324[a]	54[a]
>PC50	379	423[a]	0[a]	22[a]	259[a]	43[a]
Maternal Omega-6 /Omega-3 intake ratio during pregnancy						
<=PC50	1003	585[a]	72[a]	56[a]	343[a]	56[a]
>PC50	992	352[a]	0[a]	0[a]	231[a]	41[a]
Maternal 25(OH)D3 plasma levels (ng/ml) pregnancy.						
<=PC50	957	457	40	21[a]	287	50
>PC50	949	457	43	31[a]	284	47
Maternal iodine urine levels (mc/gl) pregnancy.						
<=PC50	842	445	30[a]	25	283	49
>PC50	869	463	46[a]	29	286	47

[a]P-value<0.10. [b]Wilcoxon rank-sum test. PC50=50th Percentile.

WEB TABLE 3

Maternal pregnancy first trimester seafood consumption by child neuropsychological outcomes[b] (INMA Study, 2004-2008)

		Seafood Intake, g/week				
Child outcomes	N	Total (50th Percentile)	Large Fatty Fish (50th Percentile)	Smaller Fatty Fish (50th Percentile)	Lean Fish (50th Percentile)	Shellfish (50th Percentile)
Bayley Mental Scale (14 m) (median, rank: 99, 49-151)						
<=PC50	993	445	41	23	274	48
>PC50	991	461	44	28	290	49
McCarthy General Cognitive Scale (5 y) (101, 35-150)						
<=PC50	831	435[a]	39[a]	19[a]	270[a]	49
>PC50	812	472[a]	48[a]	31[a]	293[a]	50
CAST, Childhood Asperger Syndrome Test (5 y) (6, 0-24)						
<=PC50	848	460[a]	44	25	282	50
>PC50	606	434[a]	46	11	265	49

[a]P-value<0.10. [b]Wilcoxon rank-sum test. M=Months. Y=Years. PC50=50th Percentile.

WEB TABLE 4

Associations between maternal pregnancy first trimester seafood consumption and child Bayley Motor Scale at 14 months (INMA Study, 2004-2008)

Seafood intake (g/w)[e]	N subjects	Fully adjusted[a]	
		β Coeff	95% CI
Total seafood consumption (10 g/w)[f]	1892	-0.01	-0.03, 0.02
Quantiles: 1[b]	383	0.00	Referent
2	392	2.42[d]	0.27, 4.57
3	364	0.51	-1.69, 2.71
4	386	0.07	-2.12, 2.27
5	367	0.92	-1.30, 3.13
p-for-trend		*0.895*	
Large fatty fish (10 g/w)[f]	1892	0.02	-0.06, 0.09
Quantiles: 1	853	0.00	Referent
2	341	-0.46	-2.39, 1.47
3	345	1.56	-0.38, 3.50
4	353	-0.13	-2.07, 1.82
p-for-trend		*0.93*	
Small fatty fish (10 g/w)[f]	1892	0.05	-0.03, 0.14
Quantiles: 1	877	0.00	Referent
2	333	-0.58	2.60, 1.44
3	338	-0.26	-2.19, 1.67
4	344	1.55	-0.37, 3.47
p-for-trend		*0.139*	
Lean fish (10 g/w)[f]	1892	-0.02	-0.05, 0.02
Quantiles: 1	387	0.00	Referent
2	386	1.67	-0.47, 3.82
3	380	2.50[d]	0.30, 4.69
4	372	-0.62	-2.81, 1.57
5	367	1.10	-1.13, 3.34
p-for-trend		0.99	
Shellfish (10 g/w)[f]	1892	0.04	-0.07, 0.16
Quantiles: 1	373	0.00	Referent
2	370	-1.34	-3.56, 0.88
3	384	-0.75	-2.93, 1.42
4	394	0.25	-1.92, 2.41
5	371	1.02	-1.23, 3.26
p-for-trend		*0.156*	

[a]Regression models adjusted for: gender of the child, cohort, and quality of the test, child birth weight, gestational age, duration of breastfeeding, child age during testing, maternal age, energy intake (Kcal) during pregnancy, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [b]Results were similar when reference group included all mothers eating ≤ of 340 g per week (data not shown). [c]P-value<0.10. [d]P-value<0.05. [e]Median of total seafood intake per each Quantile (Q), g per week: Q1=195g; Q2=338g; Q3=461g; Q4=600g; Q5=854g. Median of large fatty fish intake: Q1=None; Q2=48g; Q3=92g; Q4=238g. Median of small fatty fish intake: Q1=None; Q2=37g; Q3=69g; Q4=147g. Median of lean fish intake: Q1=90g; Q2=192g; Q3=286g; Q4=382g; Q5=557g. Median of shellfish intake: Q1=None; Q2=27g; Q3=49g; Q4=76g; Q5=139g. [f]10g/week increase. CI=Confidence Interval. W=Week.

WEB TABLE 5

Associations between maternal pregnancy first trimester total seafood consumption and child Bayley Scale at 14 months and McCarthy Scale and CAST at 5 years (reference group ≤ 340g; INMA Study, 2004-2008)

Seafood intake (g/w)[d]	N subjects	Fully adjusted[a] β Coeff	Fully adjusted[a] 95% CI
Total seafood consumption		Mental Bayley Scale (14 m)	
Quantiles: 1	592	0.00	Referent
2	183	2.23[b]	-0.28, 4.74
3	364	0.73	-1.27, 4.71
4	386	2.33[c]	0.35, 4.30
5	367	1.51	-0.49, 3.51
p-for-trend		*0.10*	
Total seafood consumption		MSCA General Cognitive Scale (5 y)	
Quantiles: 1	496	0.00	Referent
2	164	1.81	-0.55, 4.17
3	299	1.75[b]	-0.18, 3.69
4	323	2.46[c]	0.56, 4.36
5	308	1.71[b]	-0.23, 3.65
p-for-trend		*0.057*	
Total seafood consumption		Childhood Asperger Syndrome Test (CAST) (5 y)	
Quantiles: 1	446	0.00	Referent
2	140	-0.03	-0.60, 0.54
3	280	-0.25	-0.70, 0.21
4	284	-0.41[b]	-0.86, 0.05
5	262	-0.34	-0.81, 0.12
p-for-trend		*0.078*	

[a]Regression models adjusted for: gender of the child, cohort, quality of the test, child birth weight, gestational age, duration of breastfeeding, child age during testing, maternal age, energy intake (Kcal) during pregnancy, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [b]P-value<0.10. [c]P-value<0.05. [d]Median of total seafood intake per each Quantile (Q), g per week: Q1=243g; Q2=370g; Q3=461g; Q4=600g; Q5=854g. CI=Confidence Interval. W=Week. M=Month. Y=Year.

WEB TABLE 6

Associations between maternal pregnancy third trimester seafood consumption and McCarthy Scales at 5 years (INMA Study, 2004-2008)

Seafood intake (g/w)[d]	N subjects	Fully adjusted[a] General Cognitive		Fully adjusted[a] Perceptive-Performance	
		β Coeff	95% CI	β Coeff	95% CI
Total seafood consumption (10 g/w)[e]	1567	0.01	-0.01, 0.04	0.02	-0.00, 0.04
Quantiles: 1	324	0.00	Referent	0.00	Referent
2	320	-1.41	-3.47, 0.66	-0.67	-2.78, 1.43
3	298	-0.68	-2.81, 1.45	-0.17	-2.34, 2.00
4	316	-0.29	-2.40, 1.83	2.09[b]	-0.06, 4.24
5	309	0.73	-1.39, 2.86	1.34	-0.81, 3.51
p-for-trend		*0.23*		*0.04*	
Large fatty fish (10 g/w)[e]	1567	0.07[c]	0.00, 0.14	0.07[c]	0.00, 0.14
Quantiles: 1	652	0.00	Referent	0.00	Referent
2	290	1.29	-0.62, 3.19	0.83	-1.11, 2.77
3	313	1.49	-0.38, 3.36	2.21[c]	0.30, 4.12
4	310	1.89[b]	-0.04, 3.82	1.99[c]	0.02, 3.95
p-for-trend		*0.06*		*0.04*	
Small fatty fish (10 g/w)[e]	1567	-0.03	-0.11, 0.06	0.00	-0.09, 0.09
Quantiles: 1	678	0.00	Referent	0.00	Referent
2	259	-0.70	-2.67, 1.26	-1.20	-3.21, 0.80
3	338	-0.41	-2.18, 1.36	0.18	-1.62, 1.99
4	290	0.21	-1.63, 2.05	0.67	-1.21, 2.54
p-for-trend		*0.88*		*0.43*	
Lean fish (10 g/w)[e]	1567	0.01	-0.03, 0.05	0.02	-0.02, 0.06
Quantiles: 1	332	0.00	Referent	0.00	Referent
2	319	-0.54	-2.61, 1.52	-1.03	3.13, 1.08
3	305	0.35	-1.76, 2.46	0.54	-1.61, 2.69
4	313	0.08	-2.04, 2.20	0.80	-1.35, 2.95
5	296	0.54	-1.64, 2.71	0.72	-1.49, 2.94
p-for-trend		*0.50*		*0.22*	
Shellfish (10 g/w)[e]	1567	-0.07	-0.19, 0.04	-0.07	-0.19, 0.04
Quantiles: 1	301	0.00	Referent	0.00	Referent
2	308	0.23	-2.07, 2.53	-0.26	-2.60, 2.08
3	320	-1.33	-3.46, 0.81	-1.27	-3.44, 0.90
4	320	0.00	-2.11, 2.12	0.52	-1.63, 2.68
5	316	-1.53	-3.67, 0.60	-1.73	-3.91, 0.44
p-for-trend		*0.17*		*0.19*	

[a]Regression models adjusted for: gender of the child, cohort, quality of the test, child birth weight, gestational age, duration of breastfeeding, child age during testing, maternal age, energy intake (Kcal) during third trimester of pregnancy, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [b]P-value<0.10. [c]P-value<0.05. [d]Median of total seafood intake per each Quantile (Q), g per week: Q1=196g; Q2=337g; Q3=455g; Q4=585g; Q5=828g. Median of large fatty fish intake: Q1=None; Q2=45g; Q3=93g; Q4=253g. Median of small fatty fish intake: Q1=None; Q2=41g; Q3=72g; Q4=142g. Median of lean fish intake: Q1=97g; Q2=193g; Q3=267g; Q4=390g; Q5=561g. Median of shellfish intake: Q1=None; Q2=24g; Q3=46g; Q4=73g; Q5=133g. [e]10g/week increase. CI=Confidence Interval. W=Week.

WEB TABLE 7

Fully adjusted[a] associations between maternal pregnancy first trimester seafood consumption and McCarthy Scales at 5 years by location (Cantabric Sea vs. Mediterranean Sea; INMA Study, 2004-2008)

Seafood intake (g/w)	N subjects	Cantabric General Cognitive[a]		N subjects	Mediterranean General Cognitive[a]	
		β Coeff	95% CI		β Coeff	95% CI
Total seafood consumption (10 g/w)[d]	671	0.02	-0.01, 0.06	919	0.03	-0.01, 0.07
Quantiles: 1	98	0.00	Referent	222	0.00	Referent
2	128	04	-3.61, 3.70	212	2.30[b]	-0.20, 4.79
3	128	1.23	-2.45, 4.92	171	2.73[c]	0.07, 5.39
4	160	3.84[c]	0.32, 7.35	163	1.89	-0.82, 4.61
5	157	1.09	-2.44, 4.61	153	3.46[c]	0.68, 6.23
p-for-trend		*0.25*			*0.03*	
Large fatty fish (10 g/w)[d]	671	0.08	-0.02, 0.17	919	0.07	-0.03, 0.17
Quantiles: 1	229	0.00	Referent	475	0.00	Referent
2	114	-0.24	-3.39, 2.91	171	2.81[c]	0.47, 5.15
3	146	1.09	-1.84, 4.03	150	2.08[b]	-0.39, 4.54
4	182	2.00	-0.75, 4.75	122	2.22	-0.45, 4.88
p-for-trend		*0.12*			*0.07*	
Small fatty fish (10 g/w)[d]	671	0.06	-0.06, 0.18	919	-0.09[b]	-0.20, 0.01
Quantiles: 1	337	0.00	Referent	399	0.00	Referent
2	101	-1.80	-5.06, 1.45	179	1.07	-1.36, 3.50
3	106	0.78	-2.27, 3.84	182	1.29	-1.06, 3.65
4	127	1.30	-1.56, 4-15	158	0.58	-1.92, 3.07
p-for-trend		*0.32*			*0.54*	
Lean fish (10 g/w)[d]	671	0.00	-0.05, 0.06	919	0.07[c]	0.02, 0.1
Quantiles: 1	82	0.00	Referent	246	0.00	Referent
2	123	1.20	-2.70, 5.10	202	1.42	-1.05, 3.90
3	149	2.36	-1.42, 6.15	173	1.19	-1.43, 3.82
4	139	1.79	-2.05, 5.64	168	2.98[c]	0.36, 5.61
5	178	0.98	2.70, 4.67	129	3.16[c]	0.30, 6.02
p-for-trend		*0.89*			*0.01*	
Shellfish (10 g/w)[d]	671	0.06	-0.11, 0.23	919	-0.06	-0.21, 0.09
Quantiles: 1	145	0.00	Referent	162	0.00	Referent
2	119	-0.66	-4.05, 2.73	188	-0.56	-3.39, 2.26
3	139	2.40	-0.85, 5.64	192	-0.58	-3.37, 2.21
4	140	3.09[b]	-0.13, 6.31	192	-1.20	-4.00, 1.60
5	128	1.92	-1.43, 5.28	184	-2.63[b]	-5.52, 0.26
p-for-trend		*0.12*			*0.06*	

[a]Regression models adjusted for: gender of the child, quality of the test, child birth weight, gestational age, duration of breastfeeding, child age during testing, maternal age, energy intake (Kcal) during pregnancy, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [b]P-value<0.10. [c]P-value<0.05. [d]10g/week increase. CI=Confidence Interval. W=Week.

WEB TABLE 8 Associations between maternal pregnancy first trimester seafood consumption and child cognitive development (McCarthy General Cognitive Scale) at 5 years, adjustments for Hg and LCPUFA concentrations in cord blood (INMA Study, 2004-2008)

Seafood intake (g/w)	N subjects	Fully adjusted[a]		Fully adjusted[a] + Hg adjusted		N subjetcs	Fully adjusted[a]		Fully adjusted[a] + LCPUFA adjusted	
		β Coeff	95% CI	β Coeff	95% CI		β Coeff	95% CI	β Coeff	95% CI
Total seafood consumption (10 g/w)[e]	1221	0.01	-0.01, 0.04	0.00	-0.03, 0.03	611	0.03*	-0.01, 0.07	0.03	-0.01, 0.07
Quantiles: 1	241	0.00	Referent	0.00	Referent	131	0.00	Referent	0.00	Referent
2	249	1.06	-1.31, 3.42	0.56	-1.82, 2.93	115	0.01	-3.25, 3.28	-0.27	-3.53, 2.98
3	240	2.16[c]	-0.25, 4.56	1.22	-1.25, 3.69	115	2.60	-0.69, 5.89	2.14	-1.15, 5.44
4	262	2.59[d]	0.22, 4.95	1.71	-0.71, 4.13	125	3.19[d]	-0.03, 6.42	2.92[c]	-0.29, 6.14
5	230	1.06	-1.31, 3.42	0.15	-2.36, 2.66	125	1.77	-1.49, 5.03	1.34	-1.92, 4.60
p-for-trend		*0.30*		*0.80*			*0.13*		*0.19*	
Large fatty fish (10 g/w)[e]	1221	0.05	-0.03, 0.13	0.03	-0.06, 0.11	611	0.06	-0.06, 0.18	0.04	-0.08, 0.16
Quantiles: 1	535	0.00	Referent	0.00	Referent	281	0.00	Referent	0.00	Referent
2	218	1.98[c]	-0.14, 4.09	1.53	-0.59, 3.66	118	4.22[d]	1.51, 7.02	3.79[d]	0.97, 6.60
3	231	1.07	-1.03, 3.18	0.53	-1.59, 2.66	105	2.66[c]	-0.33, 5.66	2.27	-0.73, 5.28
4	237	1.90[c]	-0.21, 4.00	1.31	-0.81, 3.44	107	2.72[c]	-0.26, 5.72	2.32	-0.68, 5.33
p-for-trend		*0.10*		*0.28*			*0.10*		*0.18*	
Lean fish (10 g/w)[e]	1221	0.02	-0.02, 0.06	0.00	-0.04, 0.05	611	0.06[d]	0.00, 0.11	0.05[c]	-0.00, 0.11
Quantiles: 1	243	0.00	Referent	0.00	Referent	123	0.00	Referent	0.00	Referent
2	253	2.27[c]	-0.08, 4.61	1.75	-0.60, 4.11	130	0.05	-3.16, 3.25	-0.15	-3.34, 3.05
3	238	1.19	-1.23, 3.61	0.46	-1.99, 2.92	107	0.19	-3.21, 3.59	0.06	-3.33, 3.45
4	250	2.14[c]	-0.23, 4.53	1.28	-1.15, 3.71	131	3.24[d]	0.02, 6.47	2.82[c]	-0.40, 6.05
5	237	1.72	-0.73, 4.18	0.90	-1.60, 3.40	120	2.01	-1.30, 5.33	1.82	-0.40, 5.12
p-for-trend		*0.29*		*0.72*			*0.07*		*0.10*	

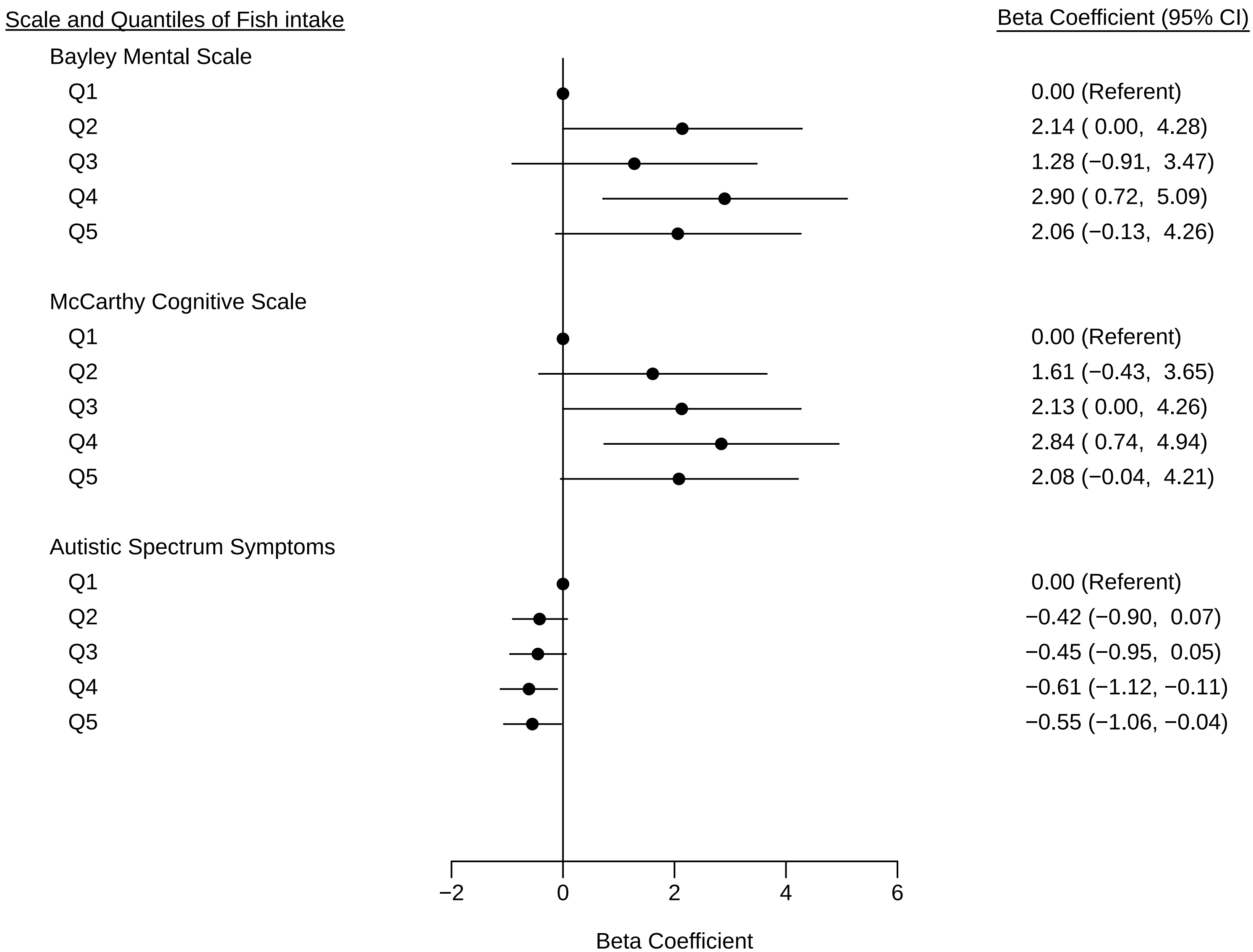
[a]Regression models adjusted for: gender of the child, cohort, quality of the test, child birth weight, gestational age, duration of breastfeeding, child age during testing, maternal age, energy intake (Kcal) during pregnancy, education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [b]Results were the similar when reference group included all mothers eating ≤ of 340 g per week (Re. group n=496; data not shown). [c]P-value<0.10. [d]P-value<0.05. MSCA memory scale remained associated with total seafood consumption after Hg adjustment (Q4=2.82, 0.20 to 5.43). Hg and LCPUFA levels showed positive associations with the outcome (data not shown). [e]10g/week increase. CI=Confidence Interval. W=Week.

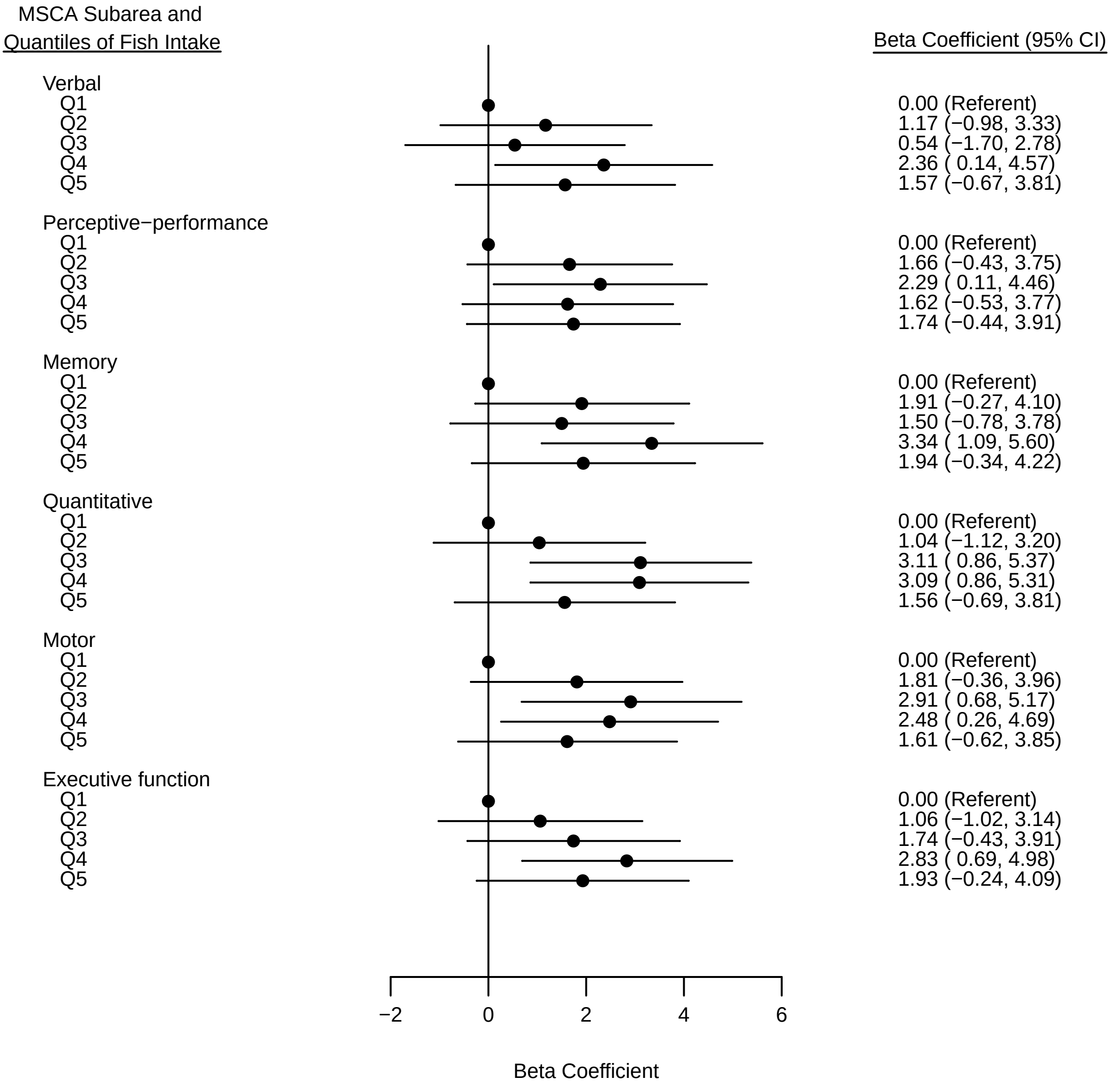
WEB TABLE 9

Associations between child seafood consumption and McCarthy General Cognitive Scale at 5 years old (INMA Study, 2004-2008)

Seafood intake (g/w)[e]	N subjects	Fully adjusted[a]		Fully adjusted[a] + maternal seafood consumption[b]	
		β Coeff	95% CI	β Coeff	95% CI
Total seafood consumption (10 g/w)[f]	1521	0.06[c]	-0.00, 0.12	0.05	-0.01, 0.11
Quantiles: 1	309	0.00	Referent	0.00	Referent
2	289	0.48	-1.66, 2.62	0.44	-1.70, 2.58
3	319	0.61	-1.50, 2.73	0.31	-1.81, 2.43
4	308	0.80	-1.34, 2.95	0.49	-1.66, 2.65
5	296	1.81	-0.35, 3.98	1.43	-0.75, 3.62
p-for-trend		*0.096*		*0.158*	
Large fatty fish (10 g/w)[f]	1521	0.14	-0.04, 0.31	0.14	-0.03, 0.31
Quantiles: 1	690	0.00	Referent	0.00	Referent
2	458	0.03	-1.52, 1.63	-0.11	-1.72, 1.49
3	373	1.90[d]	0.18, 3.61	1.68[c]	-0.05, 3.41
p-for-trend		*0.039*		*0.056*	
Small fatty fish (10 g/w)[f]	1521	-0.13	-0.35, 0.09	-0.13	-0.34, 0.09
Quantiles: 1	867	0.00	Referent	0.00	Referent
2	331	0.46	-1.26, 2.19	0.39	-1.33, 2.12
3	330	-0.30	-2.07, 1.46	-0.39	-2.16, 1.38
p-for-trend		*0.85*		*0.79*	
Lean fish (10 g/w)[f]	1521	0.09[d]	0.01, 0.17	0.08[d]	0.00, 0.16
Quantiles: 1	300	0.00	Referent	0.00	Referent
2	303	1.04	-1.09, 3.17	0.97	-1.17, 3.09
3	315	-0.29	-2.42, 1.83	-0.52	-2.65, 1.62
4	314	2.59[d]	0.46, 4.72	2.29[d]	0.14, 4.44
5	296	1.87[c]	-0.30, 4.05	1.71	-0.49, 3.90
p-for-trend		*0.041*		*0.061*	
Shellfish (10 g/w)[f]	1521	0.01	-0.23, 0.25	0.01	-0.23, 0.25
Quantiles: 1	422	0.00	Referent	0.00	Referent
2	391	0.56	-1.37, 2.45	0.55	-1.37, 2.46
3	372	0.92	-0.96, 2.79	0.97	-0.92, 2.86
4	343	0.63	-1.31, 2.57	0.66	-1.31, 2.63
p-for-trend		*0.51*		*0.46*	

[a]Regression models adjusted for: gender of the child, cohort, quality of the test, child birth weight, gestational age, duration of breastfeeding, child age during testing, maternal age, child energy intake (Kcal), maternal education level and social class, pre-pregnancy BMI, parity and country of origin/birth. [b]The same regression models additionally adjusted for the corresponding maternal seafood consumption and energy intake during first trimester of pregnancy. Otherwise, maternal intakes adjusted for child intakes showed similar results (data not shown). [c]P-value<0.10. [d]P-value<0.05. [e]Median of total seafood intake per each Quantile (Q), g per week: Q1=84g; Q2=162g; Q3=213g; Q4=271g; Q5=377g. Median of large fatty fish intake: Q1=None; Q2=30g; Q3=68g. Median of small fatty fish intake: Q1=None; Q2=27g; Q3=56g. Median of lean fish intake: Q1=51g; Q2=88g; Q3=140g; Q4=183g; Q5=247g. Median of shellfish intake: Q1=None; Q2=15g; Q3=31g; Q4=61g. [f]10g/week increase. CI=Confidence Interval. W=Week.


Scale and Quantiles of Fish intake
Beta Coefficient (95% CI)
Bayley Mental Scale
Q1 0.00 (Referent)
Q2 2.14 (0.00, 4.28)
Q3 1.28 (−0.91, 3.47)
Q4 2.90 (0.72, 5.09)
Q5 2.06 (−0.13, 4.26)
McCarthy Cognitive Scale
Q1 0.00 (Referent)
Q2 1.61 (−0.43, 3.65)
Q3 2.13 (0.00, 4.26)
Q4 2.84 (0.74, 4.94)
Q5 2.08 (−0.04, 4.21)
Autistic Spectrum Symptoms
Q1 0.00 (Referent)
Q2 −0.42 (−0.90, 0.07)
Q3 −0.45 (−0.95, 0.05)
Q4 −0.61 (−1.12, −0.11)
Q5 −0.55 (−1.06, −0.04)
−2
0
2
4
6
Beta Coefficient


MSCA Subarea and
Quantiles of Fish Intake
Beta Coefficient (95% CI)
Verbal
Q1
Q2
Q3
Q4
Q5
0.00 (Referent)
1.17 (−0.98, 3.33)
0.54 (−1.70, 2.78)
2.36 (0.14, 4.57)
1.57 (−0.67, 3.81)
Perceptive−performance
Q1
Q2
Q3
Q4
Q5
0.00 (Referent)
1.66 (−0.43, 3.75)
2.29 (0.11, 4.46)
1.62 (−0.53, 3.77)
1.74 (−0.44, 3.91)
Memory
Q1
Q2
Q3
Q4
Q5
0.00 (Referent)
1.91 (−0.27, 4.10)
1.50 (−0.78, 3.78)
3.34 (1.09, 5.60)
1.94 (−0.34, 4.22)
Quantitative
Q1
Q2
Q3
Q4
Q5
0.00 (Referent)
1.04 (−1.12, 3.20)
3.11 (0.86, 5.37)
3.09 (0.86, 5.31)
1.56 (−0.69, 3.81)
Motor
Q1
Q2
Q3
Q4
Q5
0.00 (Referent)
1.81 (−0.36, 3.96)
2.91 (0.68, 5.17)
2.48 (0.26, 4.69)
1.61 (−0.62, 3.85)
Executive function
Q1
Q2
Q3
Q4
Q5
0.00 (Referent)
1.06 (−1.02, 3.14)
1.74 (−0.43, 3.91)
2.83 (0.69, 4.98)
1.93 (−0.24, 4.09)
−2
0
2
4
6
Beta Coefficient