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Seasonal variation in chemical composition and contaminants in European plaice (*Pleuronectes Platessa*) originated from the west-coast of Norway

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ABSTRACT

The seasonal effect on chemical composition and contaminants in European plaice (*Pleuronectes platessa*) captured in Norwegian waters was investigated in three seasons (September, December, April). Fillets were compared by analysing proximate composition, lipid and protein profile, including fatty acids, lipid class distribution, free and total amino acids. Trace elements and polychlorinated biphenyls (PCB) were determined to assess the potential health risk of consumption. Results on proximate composition reveal differences in water, ash, lipid, and protein content between the seasons. Beneficial levels of n3 fatty acids, including a sufficient n3/n6 ratio (6.1–8.7), were observed. Phospholipids and cholesterol were negatively correlated with total lipid content, adding to high triacylglycerol concentrations observed in December samples. No elevated levels for toxic trace elements, yet detrimental concentrations of dioxin-like PCB-118 were found. Results show that European plaice is highly nutritious with desirable amino and fatty acid profile throughout the year, and with few potential risks.

1. Introduction

European plaice *(Pleuronectes platessa)* has been of great commercial interest in Europe for the past decades, with its leading capture being the Northern Sea (EC, 2020). The Netherlands hold a significant part of the flatfish capture, followed by Denmark, United Kingdom, Belgium, France and Germany (EC, 2020). The Annual Economic Report on the EU Fishing Fleet of 2020 states that it is expected that stocks of European plaice will decrease in the Northern Sea as fish move further north (EC, 2020). With these changes in migration patterns, increased stock volumes of European plaice in the Norwegian sea and evolving significance for Norwegian fisheries can be expected in the future. Data on catch and quota collected from the Norwegian Directorate of Fisheries shows slight increases from 2020 to 2021 of 699 to 794 tons (whole fish equivalent) for European plaice captured in Norwegian waters (Directorate of Fisheries, 2022). Data on human consumption of plaice in Norway is limited, as consumer studies only include regularly consumed fish.

Seafood is known to provide significant amounts of bioavailable nutrients like proteins, lipids, and micronutrients such as vitamins, iron, selenium, or zinc with well-recognized health benefits (Ahern et al., 2021; EFSA, 2014). The recommended intake for seafood, including fish, ranges from 100 g (FAO/WHO, 2011) to 300 g (EFSA, 2014) per week to ensure a satisfying intake of certain nutrients. Fish is a good source of long-chain polyunsaturated fatty acids (LC-PUFAs), supporting human health by various metabolic functions. Next to providing essential fatty acids, fish delivers considerable amounts of proteins. With its excellent digestibility and sufficient distribution of essential amino acids, fish is a source of high-quality proteins. Moreover, free amino acids contribute to fish's flavour and taste development, relevant for sensory perception during consumption (Ruiz-Capillas & Moral, 2004).

The lipid and protein content of fish can vary throughout the year, as fish must manage environmental conditions fluctuations. Due to changes in nutrient content and feed availability, changes within the proximate composition of fish have been observed (Celik, 2008; Gökçe, Taşbozan, Çelik, & Tabakoğlu, 2004). Furthermore, factors such as water temperature, maturity, and sex can influence the nutritional composition and hence the fish quality (Aidos, van der Padt, Luten, & Boom, 2002). Several studies have been conducted to investigate the differences related to the proximate composition, especially focusing on distribution of fatty acids of fish and other seafood (Aidos et al., 2002;

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Gökçe et al., 2004; Küçükgülmez, Celik, Ersoy, & Yanar, 2010; Mateos, Lewandowski, & Su, 2010).

However, the likelihood of elevated levels of contaminants, such as mercury in the form of methylmercury or dioxins and polychlorinated biphenyls (PCBs), must be considered when consuming fish. Contaminants can bioaccumulate in organisms, especially in predatory fish at the top of the aquatic food chain (Costa, Korn, Brito, Ferlin, & Fostier, 2016) and demersal fish (Parolini et al., 2020). European plaice is a demersal fish species inhabiting muddy or sandy areas near the seabed (Parolini et al., 2020). According to Parolini et al. (2020), concentrations of toxins like PCBs are elevated closer to the sea bottom than in the upper sea column, increasing the risks of accumulating chemical toxins in groundfish species such as the European plaice.

Literature on the chemical composition of European plaice and its compositional changes throughout the year are limited. So far, European plaice is of no great commercial relevance in Norway with low overall catching volumes. Considering the ongoing changes in migration patterns due to climatical changes, leading to bigger stock volumes further north, European plaice will probably gain increased relevance in Norway in coming years. Therefore, this study investigated the chemical composition of European plaice captured in Norwegian coastal water. Following the fishing seasons of the Norwegian west coast with samplings in autumn (September), winter (December) and spring (April), the study aimed to identify seasonal variations within the proximate and nutritional composition, including determination of lipid classes, free amino acids, and the fatty acid profile of European plaice fillets. In addition, physiochemical quality parameters such as texture and water holding capacity (WHC) were studied on the fresh fillet. Moreover, analyses on trace elements, including toxic elements, and PCBs, were conducted to assess chemical contaminants and consequently the potential health risk of consuming European plaice captured in Norwegian waters.

2. Material and methods

2.1. Raw material

European plaice individuals were caught at three different times of the year by local fishermen on the Norwegian west coast using purse seine. The samplings took place in autumn (September 2020), winter (December 2020), and spring (April 2021). A total of 59 individuals ($n_{(sept)} = 17 \times 2$; $n_{(dec)} = 21 \times 2$; $n_{(apr)} = 21 \times 2$) were collected and muscle samples were taken from two fillets per individual ($n \times 2$) to get substantial knowledge on the chemical composition and to detect possible variations in the nutritional composition within different body parts. The upper belly fillet and bottom loin fillet were chosen, and each analysed in duplicates, giving four values per individuals. Following the FAO Major Fishing Areas (FAO, 1990–2021), the capture took place in area 2.a.2. with varying sampling locations in this area. The water temperature at catch was 13 °C, 8.7 °C and 5.4 °C, respectively. The pH was measured before the fish was gutted, weighed, and thereafter stored as a whole on ice until the end of rigor mortis. The average pH value of gutted fish was between 6.6 and 6.8 in all three seasons and gutted fish weighed 0.91 ± 0.29 kg (September), 1.17 ± 0.36 kg (December) and 0.84 ± 0.33 kg (April). The fish were filleted 41 to 57 h post-mortem, depending on the release of rigor mortis. For physiochemical analyses, fresh fillets were used and for chemical analyses, fillets were immediately frozen and stored at –80 °C. Parts of the samples were freeze-dried for further analyses on total amino acid distribution, as well as trace elements and polychlorinated biphenyls.

2.2. Proximate composition

Dry matter and ash content were determined gravimetrically. For dry matter analysis, 2–3 g wet weight (ww) samples were dried for 24 h at 105 °C (ISO:6496, 1999). To determine the inorganic matter, the samples were put in a muffle furnace at 550 °C for 24 h. Samples were weighed before and after heating.

Total lipids (%) were determined as described by Bligh and Dyer (1959). Around 2 g (ww) sample was weighed into chloroform-stable tubes. To achieve phase separation, thorough homogenizing and centrifugation were applied on the solvent-sample mixture. The chloroform-lipid phase was separated from the aqueous phase. Chloroform was evaporated with nitrogen, and the total amount of lipids was calculated (Bligh and Dyer, 1959). Additional oil extracts were kept, to investigate the lipid distribution and lipid classes.

Total crude protein (%) was determined by a Kjeldahl apparatus (K-449 and K-375. Büchi Switzerland) following the application manual No. 114/2013 of Büchi by applying 15 mL sulfuric acid H_2SO_4 and two titanium tablets (3.5 g K_2SO_4/0.105 g $CuSO_4 \cdot 5H_2O$/0.105 g TiO_2) for digestion of the samples. For titration, boric acid H_3BO_3 was used, and a conversion factor of 6.25 × Nitrogen (%) was applied according to NMKL (2003) to calculate the total protein content.

2.3. Lipid profile

2.3.1. Fatty acid composition

To determine the fatty acid composition, six individuals per catching season (in total 18 samples) were analysed and thereof approximately 100 mg of extracted oil per sample was used to prepare fatty acid methyl esters (FAMEs). To obtain FAMEs, the method of Metcalfe, Schmitz, and Pelka (1966) was followed. Hence 3 mL KOH (0.5 M) was added to the sample tubes and samples heated up to 60–80 °C for 20 min, with mixing in between. After cooling down, 5 mL of methanolic BF_3 was added, and samples were heated for another 5 min before 2 mL of *n*-butylacetat was added, tubes filled up with saturated NaCl (~1.5 mL) and 1–2 scoops of sodium sulfate were added. Finally, 200 µL Hexane was added. The organic phase containing the lipid fraction was transferred and used to detect the fatty acid composition with gas chromatography. A FAME mixture (Supelco 37 Component FAME Mix, Merck Life Sciences AS, Oslo, Norway) composed of 37 fatty acid methyl esters was used as an external standard.

Fatty acid methyl ester analyses were performed on a gas chromatography (GC) apparatus (Agilent 6850, Agilent Technologies, Santa Clara, USA), equipped with a polyethylene glycol column (HP-INNOWAX, i.D.: 250 µm; film: 0.25 µm), a flame ionization detector (FID) adjusted to 310 °C and an evaporation injector (inlet: 260 °C, pressure: 18.1 psi). Hydrogen was used as carrier gas. The oven program was set to a constant temperature of 160 °C for 3 min, with an increase of 3 °C/min to 240 °C and held for 3 min.

The identification of fatty acids was based on the relative retention times (RRTs) of the external FAME standards. Chromatogram peaks were considered for determination, when the retention time of the peak matched the peak of the external standard compound. The percentage distribution of each individual fatty acid was determined by calculating the intensity of each specific peak against the total intensity of FAMEs in each specific sample.

2.3.2. Lipid class distribution

Lipid classes were analysed on three pooled samples per season (in total 9 samples). Each pooled sample contained the oil fraction of three individuals to guarantee a representative size of the sample population per season as well as to provide enough oil for analysis. The extracted oil phase as described in 2.2 was used, chloroform was evaporated with liquid nitrogen using a heating block (Stuart™ block heater type: SBH130D/3; Cole-Parmer, United States) set to 40 °C equipped with a sample concentrator (Stuart™ concentrator type: SBHCONC/1; Cole-Parmer, United States) and thereafter samples got mixed. The prepared samples were analysed externally by Innolipids AS, Norway with an HPLC system coupled to an ELSD detector and equipped with a ReproSil-Pur CN column (i.D.: 4.6 mm; length: 200 mm; particle size: 3 µm; Dr Maisch GmbH, Germany). A mixture of polar and non-polar

eluents was chosen and a flow rate of 1.0 mL/min was applied. The samples were analysed for phospholipids (PL), lyso-PL, cholesterol, cholesterol-esters, free fatty acids (FFA), monoacylglycerols (MAG), diacylglycerols (DAG) and triacylglycerols (TAG). Polar lipids (phosphatidylcholine and phosphatidylethanolamine) were quantified from a standard curve prepared from a herring roe PL concentrate. Refined moisture-free cod liver oil was used to prepare the standard curve for TAG.

2.4. Protein profile

2.4.1. Amino acid distribution

The amino acid profile analysis was done as described previously by Blackburn (1978). A total of 17 essential and non-essential amino acids, excluding tryptophan, which is destroyed during acid hydrolysis, are detected with this method. The freeze-dried muscle samples (50 mg protein) were hydrolysed with 1 mL 6 M HCl at 105 °C for 22 h. The samples got neutralized through titration using NaOH. The solutions were filtered through glass microfiber filters (GF/C) applying suction, diluted, and filtered through a 0.2 μm polyethersulfone filter (VWR International; United States), before analysed on an ultra-HPLC (UltiMate 300, Thermo Scientific).

The HPLC system included a TSP P400 pump, ultimate 3000WP injector, RF2000 detector, and a Nova-Pak C18 column (WAT086344, particle size: 4 μm, 3.9 mm*150 mm, Waters Corp., USA). Methanol and 0.08 M Sodium acetate with 2 % tetrahydrofuran were applied as mobile phases. The system's flow rate was adjusted to 0.9 mL/min.

2.4.2. Free amino acid distribution

The free amino acid profile was determined according to Osnes and Mohr (1985). A water-soluble protein extract (2 g ww in 10 mL) was prepared by homogenizing and centrifuging muscle samples in distilled water. 1 mL extract was transferred to a microcentrifugation tube, and 0.25 mL 10 % sulphosalisylic acid was added. The samples were shaken and left at + 4 °C for 30 min before centrifuging for 10 min. The supernatant was diluted and filtered through a 0.2 μm polyethersulfone filter (VWR International; North America) before analyses were done on an ultra-HPLC (UltiMate 300, Thermo Scientific). The same system parameters were applied as in 2.4.1.

2.5. Physiochemical characteristics

Water holding capacity (WHC) was measured according to Skipnes, Østby, and Hendrickx (2007) on the fresh upper belly fillets of chosen individuals. The analysis was performed by punching each fillet three times with a round sharp cutting tool (diameter: 31 mm). Two pieces were weighed and placed into a metal carrier (Part No.4750. Hettich Lab Technology, Germany) and centrifuged with 1800 rpm (4 °C, 15 min). The third portion was weighed and dried to analyse dry matter, hence water content (WC) at 105 °C for 24 h. WHC was calculated using following equations from Skipnes et al. (2007): $WHC = \left(\frac{(W-\Delta W)}{W}\right) \times 100\%$, where $W = \left(\frac{m_W}{(m_W+m_D)}\right) \times 100\%$ and $\Delta W = \left(\frac{\Delta m_W}{(m_W+m_D)}\right) \times 100\%$. Where m_W being the mass of water, m_D the mass of dry matter and Δmv being the separated water in the sample, being removed during centrifugation.

Texture Profile Analysis (TPA) was performed on fresh fillets with skin by a puncture test using a Texture Analyser (TA-XT Plus, Stable Micro Systems, UK), including a 5 kg load cell. The texture was determined by poking the fillets three times using a flat-ended cylindrical probe (diameter 25 mm, P/0.5). The muscle samples were compressed to 60 % of fillet thickness (F60), measuring the fillet firmness. Moreover, breaking force (Bf), being the force (N) employed until the breakage of the fillet's surface, was detected. The data analysis was carried out with the texture analysis software Exponent (www.stablemicrosystems, Stable Miro Systems ltd.).

2.6. Trace elements and polychlorinated biphenyls

For analyses on trace elements and polychlorinated biphenyls (PCBs), the fish were pooled together representing different catching locations and seasons. Each sample contained three individuals of same size, equally distributed and homogenized. In total, two pooled samples per season (in total 6 samples) were analysed for trace elements including toxic elements and PCBs.

2.6.1. Trace elements

Samples were analysed for Zn, V, Se, Pb, Ni, Mo, Mn, Hg, Fe, Cu, Cr, Co, Cd, As, and Ag using an 8800 Triple Quadrupole inductive coupled plasma mass spectrometry (ICP-MS) system (Agilent, USA) equipped with a prepFAST M5 autosampler (ESI, USA). Accuracy of the analysis was determined using certified reference materials cod (MODAS-5, CodTis: Nr. 0496) and herring tissues (MODAS-3, HerTis: Nr. 0958). Each sample, containing 400 mg freeze-dried muscle and 5 mL 50 % nitric acid (HNO_3 v/v), was decomposed in a high-pressure microwave digestion reactor (UltraClave, Milestone GmbH, Leutkirch,Germany) followed by dilution with ultrapure water (~18.2 MΩ·cm) to achieve a final HNO_3 concentration of 0.6 M before being analysed with ICP-MS system following methods as described by Sørmo et al. (2011). System parameters during analysis include general parameters: RF Power (1550 W), Nebulizer gas (0.75 L/min), Makeup gas (0.35 L/min), sample depth (8.0 mm), O_2 Mode: O_2 gas flow (0.675 L/min) and NH_3 mode: NH_3 gas flow (1.5 L/min), He gas flow (1.5 L/min).

2.6.2. Polychlorinated biphenyls (PCBs)

The analysis for PCBs included: PCB-3, 8, 28, 52, 101, 118, 138, 153, 180, 195, 206 and 209. The fish samples were extracted as described by Teunen et al. (2021). Approximately 0.5 g freeze-dried samples were fortified with 10 μL IS (F-PCB-mix 1 mg/L). Thereafter, the samples were extracted 2 consecutive times with 6 mL hexane: acetone (3:1, v/v). For each extraction, the mixture was ultrasonicated for 20 min, vortex mixed for 2 min, and centrifuged at 3500 rpm (Eppendorf centrifuge 5804). The supernatants were combined, and the extract was further evaporated to ~ 0.5 mL under a gentle nitrogen (N_2)-stream, purified through a 6 g acid silica (44 %, Sigma, Oslo, Norway) cartridge, from which the analytes were eluted with 20 mL hexane (MS SupraSolv®; Sigma, Oslo, Norway) followed by 15 mL dichloromethane (>99 % v v^{-1}, VWR, Oslo, Norway). The extract was evaporated to a volume of 1–2 mL using a rotary evaporator (Rota vapor R-200. Büchi Switzerland). Furthermore, the extract was evaporated to dryness under the gentle N_2-stream, redissolved in 100 μL hexane and analysed by GC–MS.

A GC–MS system (7890A, Agilent Technologies, Santa Clara, USA) with split liner injection, a Thermo TG 5MS column (length: 30 m; i.D.: 250 μm; film: 0.5 μm) and an inert mass selective detector (5975, Agilent Technologies, Santa Clara, USA) was applied to detect PCBs. The injector was set at 290 °C and the column oven temperature was programmed as follows (accounting for a total run time of 34.75 min): start temperature 50 °C held for 2 min; increased up to 250 °C at a rate of 25 °C/min and held at 250 °C for 1 min; second increase up to 286 °C at a rate of 3 °C/min and held for 3 min; third increase up to 308 °C at a rate of 8 °C/min and held for 1 min. The final temperature was reached at 310 °C at a rate of 1 °C/min and held for 3 min. The GC/MS interface was heated at 290 °C. An ionization energy was applied at 70 eV. and the electron ion source was kept at 230 °C. The MS was run in full scan (from 50 *m/z* to 550 *m/z*) as well as SIM mode. Standards were used to confirm the signal identification, while the identified target analytes of the full scan electron ionization mass spectra were compared to the available MS Library spectra (NIST MS library).

Multi-level calibration curves in the linear response interval of the detector were created for the quantification to cover the whole range of concentrations measured in the samples, and a good correlation (r^2 >

0.99) was achieved for all target analytes. The identification of analytes was based on the relative retention times to the internal standards used for quantification, the ion chromatograms and the intensity ratios of the monitored ions. The chromatographic peaks were determined when: (1) the retention time matched that of the standard compound within ± 0.05 min and (2) the signal-to-noise ratio (S/N) was higher than 3:1. For each target analyte, the limit of quantification (LOQ) was calculated as 10 times the signal from the baseline noise (S/N ratio). The quality control was performed by regular analyses of procedural blanks and by random injection of standards and solvent blanks. Recoveries for individual PCB congeners ranged between 75 and 120 % (Relative standard deviation, RSDs < 15 %).

2.6.3. Calculations on health effects of contaminants

The European Commission (EC), as well as the European Food Safety Authority (EFSA), regularly update on the maximum allowed level of specific contaminants as well as tolerable weekly intake (TWI) for safe consumption (EC, 2006, 2011; EFSA, 2009, 2012, 2014). The results obtained from section 2.6.1 and 2.6.2 were further used to evaluate whether these values exceed human intake recommendations set by the EC (EC, 2006, 2011). Furthermore, benchmark values for TWI of specific contaminants specified by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) were considered (EFSA, 2009, 2012). To determine the effect of the analysed components on a healthy adult, a person with 60 kg body weight was chosen as standard for all calculations as previously suggested by FAO/WHO (2011).

2.7. Statistical analysis

Statistical analyses were carried out using Minitab 19 (www.minitab.com, Minitab Inc., USA). A Grubbs Outlier test with a significance level of $\alpha < 0.05$ was performed to detect outliers within the data set. One-way ANOVA was applied to determine the differences between seasons and chemical parameters of the samples. A Tukey HSD post-hoc test was applied when significance was detected to investigate the differences between groups. A significance level of $\alpha < 0.05$ was chosen. A Pearson correlation was conducted between variables that were naturally dependent to each other. The correlation coefficient (r) and significant levels are indicated at the individual measurements. All analyses were carried out in 2 × 2 parallels (2 parallels per fillet; 4 in total per sample) and are presented as means ± standard deviation (SD) if not other stated.

3. Results & discussion

Fish (n = 59) was studied by analysing both the bottom loin and upper belly fillet of each individual separately. The results gave no significant differences ($P < 0.05$) between the two fillets of individual fish. Therefore, results of the two fillets were combined, generating one average value for each individual. The results shown in this section include the combined values of the two fillets.

3.1. Proximate composition and physiochemical characteristics

The analysis on proximate composition of European plaice revealed significant differences throughout the three investigated seasons as can be seen in Table 1. The lowest protein (14.17 %) and lipid (0.75 %) levels were found among fish captured in April, while the highest lipid content (1.55 %) was observed in December. The protein decreased significantly from September to April (17.64–14.17 %, $P < 0.001$), going along with an increasing water content (80.55–84.47 %, $P < 0.001$) from September to April. Furthermore, differences in the lipid ($P < 0.001$) and ash ($P < 0.001$) content were detected. Karl, Manthey-Karl, Ostermeyer, Lehmann, and Wagner (2013) studied European plaice captured in the Northern Sea between March and June. According to this study, European plaice contains about 81 % water, 16.6 % protein, 0.8 % fat and 0.9 % ash (Karl et al., 2013). In a study on common sole captured during four seasons in the Mediterranean sea, fluctuations in proximate composition were observed (Gökçe et al., 2004). Furthermore, Gökçe et al. (2004) found that common sole captured during the spawning season shows decreased lipid levels, while the protein content remained stable. Results in this study show a significantly lower lipid content in the post-spawning season in April compared to September ($P < 0.001$), however, the protein composition was also affected by spawning ($P < 0.001$). The reproductive season for European plaice from the Norwegian sea varies from North (December-May) to South (January-March) (Huse, 2018). The peak is from February to March, when fish barely feed and use up their lipid storage. This was also observed by Rijnsdorp (1989), studying European plaice in the Northern sea and reporting an average spawning peak from January to March. Dawson and Grimm (1980) describe quantitative changes in protein, lipid and energy content of European plaice carcasses (including fillet). According to Dawson et al. (1980), European plaice stops feeding from December to March, which is reflected by a constant decline of proteins and lipids, which agrees with the results in the present study. The collected data indicate that fish captured in December has built a lipid storage before entering the spawning season. Consequently, results on proximate composition in the present study show that fish captured in April have a relatively poor nutritional quality compared to the two other pre-spawning seasons. Moreover, fish captured in April were significantly lower in weight compared to December ($P = 0.006$). The high protein content and intermediate lipid level in September may further be a result of a higher water temperature (13 °C vs 8.7 °C and 5.4 °C) as well as longer day length, which has been reported to impact the feeding habits of fish (Olsson, Olsen, Carlehog, & Ofstad, 2003).

Table 1
Proximate composition of European plaice captured during three different seasons. Results presented as mean values ± SD.

Composition (%)	Seasons			P-value*
	September n = 17	December n = 21	April n = 21	
Ash	1.28 ± 0.06^{a}	1.07 ± 0.09^{c}	1.17 ± 0.09^{b}	<0.001
Water	80.55 ± 1.2^{c}	82.56 ± 1.4^{b}	84.47 ± 2.4^{a}	<0.001
Proteins	17.64 ± 0.9^{a}	15.13 ± 1.2^{b}	14.17 ± 2.4^{b}	<0.001
Lipids	1.20 ± 0.4^{a}	1.55 ± 0.6^{a}	0.75 ± 0.2^{b}	<0.001

*ANOVA was applied to detect differences in proximate composition; where significant difference was detected ($\alpha < 0.05$), a Tukey.
PostHoc test was applied. Values with different superscript (^{a,b}) within a row are significantly different ($P < 0.05$).

Furthermore, seasonal variations in physiochemical parameters have been observed in this study. Analysis of the texture and water holding capacity showed certain differences in the physiochemical quality of fillets. No significant difference was found in the WHC between the three seasons ($P = 0.059$). However, a correlation in the WHC of fillet was observed from September to April (87.8 %, 85.7 % to 80.1 %, r = -0,619, $P = 0.038$), which can be negatively correlated to higher water contents (r = -0,755, $P = 0.009$). The Bf showed identical values (approximately 50 N) for each season. The fillet's firmness was measured by compressing the samples to 60 %. The results show significant textural changes between the three seasons ($P = 0.001$). The fillets had intermediate firmness in September (59.2 N) compared to April (67.7 N). Whereas fish captured pre-spawning in December showed increased softness (45.7 N). Studies have reported that fillet texture can vary during the reproductive cycle of female fish (Aussanasuwannakul et al., 2012). Aussanasuwannakul et al. (2012) described softer fillet texture during egg development and growth, and increased firmness during spawning of rainbow trout (*Oncorhynchus mykiss*). It must be considered that relatively high standard deviations for all physiochemical parameters were observed. In this study, both male and female fish were used for experiments. Therefore, great individual differences can be

expected, explaining the discrepancy in values.

3.2. Nutritional composition

The average levels of total amino acids (total-AA) and free amino acids (free-AA) in muscle tissues of European plaice captured during three seasons are shown in Table 2. The fish contain a generous distribution of amino acids recognized as essential (EAA) for the human body. The exogenous intake of these amino acids through diet is crucial to ensure human health. Hence, European plaice can be regarded as a good source for all EAA, with a prevalence of lysine (10.6–11.3 %) and leucine (9.1–9.4 %) in all three seasons. Wu (2010) states a class of functional amino acids, which are important regulators for a variety of metabolic functions in the body. Within this group, arginine, glycine, leucine and methionine are prevalent in all three seasons. The distribution between total-EAA did not vary significantly between seasons ($P = 0.103$), however the total level of free-EAA differed ($P = 0.003$). A substantially lower level of total free-AA was found in September (59.0 mg (100) g^{-1}, $P < 0.001$) with increasing numbers from December (82.3 mg (100) g^{-1}) to April (100.1 mg (100) g^{-1}). April shows the highest amount of free-EAA, but levels fluctuated considerably between individuals in all three seasons. Histidine was the dominant free-EAA in September and lower values were found in December (P=<0.001). Free leucine, methionine and valine show similar results for September and December and are significantly lower to values found in April. This pattern was not observed in the total-AA composition. All samples contained further high concentrations of free arginine and glycine, being considerably different in all three seasons (P=<0.001). Glutamine and asparagine are found in low amounts due to the conversion to aspartic and glutamic acid during acid hydrolysis. Free-AA such as free alanine, glutamic acid and glycine are known to contribute to the distinct flavour and taste of fish and fishery products (Hayashi, Yamaguchi, & Konosu, 1981; Ruiz-Capillas et al., 2004; Wu, 2010). Karl et al. (2013) reported in average 5-fold higher total free-AA values for European plaice and other flatfish compared to this study. The level of free-AA can be elevated depending on the quality, storage conditions and freshness before freezing (Calanche et al., 2019). To guarantee freshness, fish in this study was frozen at −80 °C immediately after filleting hence explaining the relatively low free-AA levels.

The boxplots in Fig. 1 visualize the percentage distribution and variation of EAA in all three seasons. Fish caught in December shows a high variation with more extreme discrepancy in the lowest and highest measured values of isoleucine, leucine, lysine, phenylalanine, threonine, and valine. Furthermore, a bigger interquartile range was observed emphasising that fish in pre-spawning state (December samples) are heterogenous in their nutritional composition. A higher SD of the AA concentrations was observed in December compared to September and April (Table 2), probably linked to differences in the spawning state of indivuals (Huse, 2018; Rijnsdorp, 1989).

The fatty acid composition, including relevant saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids have been investigated for all three seasons (Table 3). A significantly lower concentration ($P = 0.021$) of myristic acid (C14:0) was found in September (1.63 %) compared to April, (2.96 %) while stearic acid (C18:0) showed a significantly higher value in September (3.97 %) compared to December (2.94 %, $P = 0.02$) and April (2.79 %, $P = 0.007$). High values of palmitic acid (C16:0) were found in all three seasons, which is in accordance with other findings studying flatfish species (Gökçe et al., 2004; Karl et al., 2013; Olsson et al., 2003). The percentages of the two main n3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were between 14.4 and 16.8 % and 18.2–19.3 % respectively, with no significant differences (P-EPA = 0.152, P-DHA = 0.587) between the three seasons. In a study on common sole captured during different seasons, Gökçe et al. (2004) found comparable values of DHA (18.8–20.2 %), but distinctively lower values in EPA (3.36–4.26 %) to this study. In a study of Karl et al. (2013) a comparable DHA distribution for European plaice of 19.4 % was found, whereas the reported values of 22.5 % and 1.2 % for EPA and docosapentaenoic acid (DPA) differ to the present study. DPA distributions of 2.9–3.29 % were observed in the three investigated seasons. The reported values can be considered as high concentrations for both EPA and DHA. Nevertheless, European plaice is a lean fish, composing of relatively low overall lipid levels. In addition to a beneficial n3 distribution, excellent ratios of n3/n6 (6.1–8.8) were observed, emphasising the

Table 2
Total-AA (%) and free-AA distribution (mg (100)g^{-1} ww) of European plaice during different seasons. Results presented as mean values ± SD.

	Seasons							
Amino Acids	September n = 17	December n = 21	April n = 21		September n = 17	December n = 21	April n = 21	
Essential*	Total-AA%			P-value***	Free-AA mg (100)g^{-1}			P-value***
Histidine	2.5 ± 0.3	2.8 ± 0.3	2.5 ± 0.2	0.597	7.0 ± 4.4^{A}	0.8 ± 0.4^{C}	3.7 ± 4.2^{B}	<0.001
Isoleucine	4.8 ± 0.5	5.2 ± 0.9	4.9 ± 0.3	0.450	0.9 ± 0.2^{B}	1.2 ± 0.2^{B}	1.6 ± 0.7^{A}	<0.001
Leucine	9.4 ± 1.1^{ab}	9.1 ± 2.8^{b}	9.4 ± 0.6^{a}	0.027	1.5 ± 0.3^{B}	1.8 ± 0.4^{B}	3.0 ± 1.4^{A}	<0.001
Lysine	11.3 ± 1.2^{a}	10.6 ± 3.4^{b}	11.2 ± 0.8^{a}	0.010	2.8 ± 2.2^{B}	6.6 ± 4.2^{A}	8.6 ± 3.9^{A}	<0.001
Methionine	3.5 ± 0.5^{b}	4.1 ± 0.4^{a}	3.7 ± 0.3^{a}	0.006	2.2 ± 0.9^{B}	2.0 ± 0.5^{B}	3.1 ± 1.2^{A}	<0.001
Phenylalanine	4.8 ± 0.6^{a}	4.7 ± 1.0^{a}	4.6 ± 0.3^{b}	<0.001	1.1 ± 0.2^{B}	1.4 ± 0.4^{A}	1.5 ± 0.4^{A}	0.001
Threonine	5.5 ± 0.8	5.6 ± 1.2	5.4 ± 0.4	0.247	4.5 ± 1.0	4.9 ± 2.1	5.8 ± 3.5	0.291
Valine	5.3 ± 0.6	5.4 ± 1.2	5.2 ± 0.4	0.108	1.6 ± 0.4A^{B}	1.7 ± 0.3^{B}	2.3 ± 1.0^{A}	0.001
Σ EAA*	47.2 ± 2.5	47.6 ± 8.5	46.6 ± 2.6	0.103	21.5 ± 7.4^{B}	20.5 ± 6.2^{B}	29.6 ± 12^{A}	0.003
Non-essential								
Asparagine	<0.03	<0.03	<0.03	/	<0.05^{A}	<0.03^{B}	<0.03^{B}	<0.001
Glutamine	<0.06	<0.06	<0.06	/	2.8 ± 1.0^{A}	2.8 ± 1.3^{A}	0.7 ± 0.4^{B}	<0.001
Arg/Glyc**	6.7 ± 1.0^{b}	7.1 ± 1.6^{b}	7.4 ± 0.5^{a}	0.003	14.9 ± 4.7^{C}	34.1 ± 11.5^{B}	45.9 ± 22^{A}	<0.001
Tyrosine	4.2 ± 0.6	4.5 ± 0.6	4.2 ± 0.3	0.664	1.3 ± 0.4^{A}	1.0 ± 0.3^{AB}	0.9 ± 0.4^{B}	0.005
Alanine	6.4 ± 0.9	6.5 ± 1.9	6.5 ± 0.5	0.107	10.2 ± 2.8^{B}	13.7 ± 2.9^{A}	13.2 ± 4.5^{A}	0.008
Aspartic acid	12.2 ± 1.7^{ab}	12.3 ± 2.2^{b}	12.1 ± 0.8^{a}	0.022	0.6 ± 0.2^{A}	0.4 ± 0.2^{B}	0.7 ± 0.2^{A}	<0.001
Glutamic acid	17.7 ± 2.0^{a}	16.5 ± 3.3^{b}	17.2 ± 1.1^{a}	<0.001	3.0 ± 0.9^{B}	5.4 ± 2.2^{A}	2.8 ± 1.5^{B}	<0.001
Serine	5.4 ± 0.6^{ab}	5.5 ± 1.3^{b}	5.6 ± 0.4^{a}	0.042	4.2 ± 1.1^{A}	3.6 ± 1.1^{A}	6.3 ± 2.0^{B}	<0.001
Σ NEAA	52.8 ± 3.7^{ab}	52.4 ± 9.2^{b}	53.5 ± 4.4^{a}	0.010	37.48 ± 7.7^{B}	61.9 ± 17.1^{A}	66.6 ± 26.2^{A}	<0.001
Σ Free-AA					59.0 ± 10.7^{B}	82.3 ± 22.0^{A}	100.1 ± 38.6^{A}	<0.001

*Tryptophan is not detected due to acid hydrolysis.
**Arginine/Glycine could not be separated.
***ANOVA was applied to detect differences in total AA and free AA; where significant difference was detected ($\alpha < 0.05$), a Tukey PostHoc test was applied. Values with different superscript within a row are significantly different ($P < 0.05$); small letters (a,b):total-AA; big letters (A,B): free-AA.

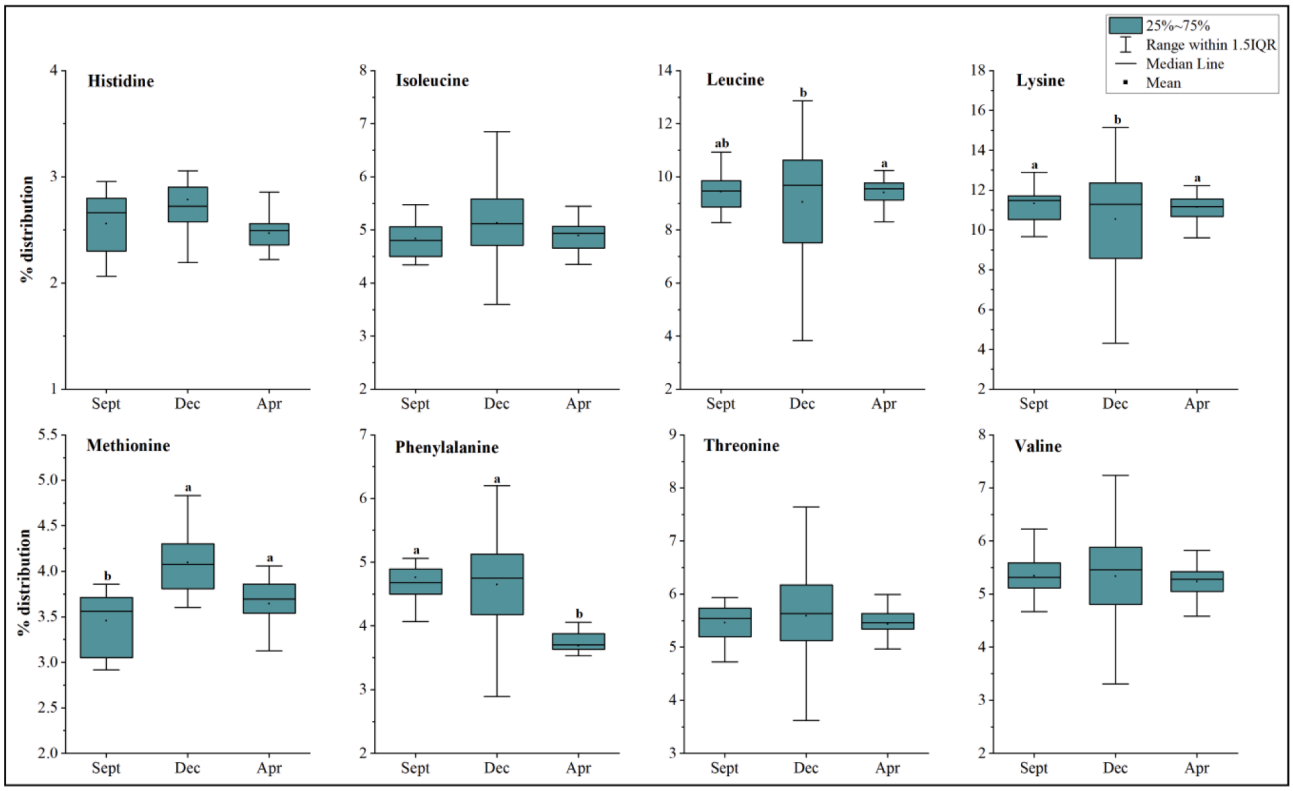

Fig. 1. Boxplots of essential amino acids (%) of European plaice captured during three different seasons (September, Sept; December, Dec; and April, Apr). Boxes visualize 25–75 % of sample values; whiskers the 1.5 interquartile range (IQR); line (median), dot (means); small letters (a,b,c) show significant differences between groups ($P < 0.05$).

importance of fish to provide sufficient levels of n3 and its role in human nutrition. Karl et al. (2013) reported a n3/n6 ratio of 4.9 for European plaice and ranges of 1.89 to 3.84 for sole were observed in the study of Gökçe et al. (2004). In the study of Olsson et al. (2003) on wild and farmed halibut (total lipid < 1 %), similar values of 40.4 % of n3 fatty acids were found, whereas values for n6 fatty acids were lower (3.5 %).

Furthermore, statistical analyses showed a negative correlation between the content of MUFA and n6 fatty acids ($r = -0.987$, $P = 0.05$). This is demonstrated by significantly lower MUFA distributions in September and April (26.08 ± 1.0 and 27.78 ± 0.7, $P = 0.001$), compared to higher levels of n6 fatty acids (6.29 ± 1.8 and 6.1 ± 1.2) in these two seasons. A significantly higher concentration ($P < 0.001$) of MUFA was found in December compared to the two other seasons. Although no significant statistical difference was found for n3 fatty acids ($P = 0.235$), a similar trend in negative correlation ($r = -0.740$) was observed. September shows the highest percentage distribution (42.15 ± 1.2), compared to the lowest MUFA portion in all three seasons. Moreover, the high concentration of PUFA in September (49.10 ± 1.3) differs considerably ($P = 0.023$) to the lower concentration in December (45.35 ± 1.1).

The analysis on lipid classes revealed abundance of three main lipid classes: phospholipids (PL), cholesterol (Chol) and triacylglycerols (TAG). Other classes such as diglycerides, monoglycerides, lysophospholipids or free fatty acids were below the limit of detection (LOD). For the three identified classes, no significant differences ($P > 0.05$) between the seasons were found. A reason for this can be the small sample size that was analysed, consisting of only three samples per season. It was not possible to analyse more samples, as the oil fraction of the fish contributed to only 0.75–1.55 % (Table 1), hence individuals needed to be merged to get enough oil for analysis. Even though no statistically significant difference ($P > 0.05$) was found, a clear trend can be seen in Fig. 2. The PL fraction (48 %) increased noticeable in April, followed with a higher cholesterol (6 %) and lower TAG (46 %) composition compared to the other two seasons. Fish in December had the highest amount of TAG. This agrees with the higher overall lipid content. TAG are storage lipids, which are used during the cold winter season to provide energy, hence fish is feeding up until winter to guarantee an adequate storage. The change in lipid class distribution can be associated with the change in total lipid concentration in the samples. The higher TAG content in December is significantly correlated to the higher total lipid content ($r = 0.988$; $P = 0.05$). With regards to total lipid content, a negative correlation has been found for the PL ($r = -0.988$; $P = 0.05$) as well as the Chol fraction ($r = -0.993$; $P = 0.039$). These correlations indicate an increase in cholesterol and phospholipids, with decreasing total lipid content. Whereas triacylglycerides are predominant at a higher total lipid content.

The amount of essential amino acids, PUFA and n3 fatty acids in the results emphasise the high nutritional value of European plaice. Moreover, the n3:n6 ratio can be regarded as very sufficient in all three studied seasons. The EAA distribution, distribution of overall PUFA as well as important n3 fatty acids such as EPA, DPA and DHA do not differ significantly between the three seasons. Differences were only observed with regards to proximate composition, showing higher lipid contents in winter, followed by low lipid and protein content in April. However, no correlation between total fat content and fatty acid distribution of SFA, MUFA or PUFA was observed in any of the three seasons. Moreover, no correlation between fatty acid composition and lipid classes was found. This indicates a relative stable composition of EAA, n3 fatty acids and total PUFA throughout the three fishing seasons.

3.3. *Trace elements and polychlorinated biphenyls*

European plaice, being a demersal fish species can accumulate higher amounts of toxic trace elements as well as contaminants like PCBs (Parolini et al., 2020). Hence the aspect of food safety must be considered when consuming European plaice regularly. Nevertheless, the

Table 3
fatty acid composition (% of total fatty acids w w^{-1}) of European plaice at different seasons. Results presented as mean values ± SD.

Fatty Acids	Seasons September n = 6	December n = 6	April n = 6	
	%	%	%	P-value**
SFA				
C14:0	1.63 ± 1.4[b]	2.71 ± 1.4[ab]	2.96 ± 0.5[a]	0.018
C15:0	0.28 ± 0.4	0.13 ± 0.2	0.34 ± 0.5	0.339
C16:0	15.86 ± 2.8	15.20 ± 2.0	14.33 ± 1.3	0.223
C17:0	0.28 ± 0.3	0.10 ± 0.2	0.13 ± 0.2	0.171
C18:0	3.97 ± 0.7[a]	2.94 ± 1.1[b]	2.79 ± 0.8[b]	0.005
Σ SFA	22.03 ± 1.0	21.08 ± 0.8	20.55 ± 0.4	0.192
MUFA				
C14:1	0.05 ± 0.2	0.01 ± 0.04	0.03 ± 0.07	0.720
C16:1 n7	5.57 ± 1.3[ab]	6.67 ± 0.7[a]	4.68 ± 2.3[b]	0.011
C17:1	0.29 ± 0.4	0.33 ± 0.4	0.34 ± 0.3	0.935
C18:1 n7	3.31 ± 2.6	2.86 ± 0.8	2.50 ± 0.6	0.480
C18:1 n9	7.66 ± 2.9[b]	10.40 ± 0.8[a]	9.37 ± 1.3[ab]	0.004
C20:1 n9	0.42 ± 0.5	0.27 ± 0.3	0.33 ± 0.3	0.651
C20:1 n11	5.50 ± 1.4	6.03 ± 0.7	5.97 ± 0.5	0.342
C22:1	3.28 ± 1.9	4.87 ± 2.0	4.6 ± 1.0	0.071
Σ MUFA	26.08 ± 1.0[b]	31.54 ± 0.6[a]	27.78 ± 0.7[b]	0.001
PUFA				
C16:2 n4	0.66 ± 0.6	0.88 ± 0.5	1.03 ± 0.4	0.216
C18:2 n6 (LA)	0.67 ± 0.6[b]	1.24 ± 0.6[a]	1.34 ± 0.6[a]	0.001
C18:3 n3	0.35 ± 0.5[b]	0.93 ± 0.6[a]	0.90 ± 0.6[a]	0.020
C18:4 n3	0.88 ± 1.0[b]	2.17 ± 1.4[a]	1.95 ± 1.0[ab]	0.023
C20:2 n6	0.14 ± 0.3	0.10 ± 0.2	0.17 ± 0.2	0.748
C20:4 n6 (AA)	5.48 ± 3.5	3.76 ± 2.4	4.41 ± 2.2	0.313
C20:4 n3	1.58 ± 2.8	0.65 ± 0.5	0.5 ± 0.5	0.254
C20:5 n3 (EPA)	16.79 ± 3.1	14.37 ± 3.6	14.75 ± 2.8	0.152
C22:5 n3 (DPA)	3.29 ± 1.1	3.04 ± 0.5	2.9 ± 0.3	0.346
C22:6 n3 (DHA)	19.27 ± 3.2	18.21 ± 2.3	18.40 ± 2.4	0.587
Σ PUFA*	49.10 ± 1.3[a]	45.35 ± 1.1[b]	46.14 ± 1.0[ab]	0.026
Σ n3	42.15 ± 1.2	39.37 ± 1.3	39.37 ± 1.1	0.108
Σ n6	6.29 ± 1.8	5.10 ± 1.2	6.1 ± 1.2	0.529
n3/n6	6.1 ± 2.5	8.8 ± 3.3	7.5 ± 3.3	0.130
Others	2.79 ± 3.5	2.03 ± 2.0	5.1 ± 5.6	0.154

*including n3 and n6.
**ANOVA was applied to detect differences in fatty acid composition; where significant difference was detected (α < 0.05), a Tukey.
PostHoc test was applied. Values with different superscript ([a,b]) within a row are significantly different (P < 0.05).

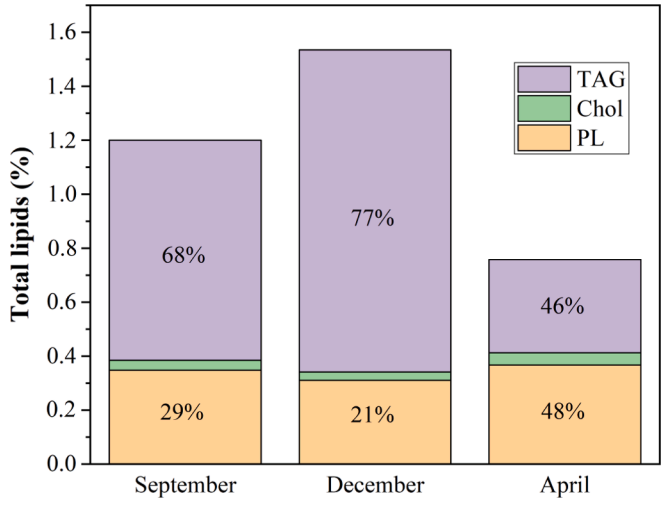

Fig. 2. Total lipid content and lipid class distribution (total distribution of 100%) of European plaice captured in September, December and April.

bioaccumulation of trace elements, including both essential elements and toxic elements depends on endogenous and exogenous factors (Afonso et al., 2013). It is possible to have higher variations of trace element concentrations between individuals of different size, sex, but also feeding ground and migration habits must be considered. In this study, different locations for the toxic screening of toxic trace elements and PCBs of fish captured during three seasons were considered. Statistical analyses have shown no significant differences (P > 0.05) between the fishing locations. It must be mentioned that the sample size for one-way ANOVA of trace elements and PCBs was rather small, as individuals were pooled together to represent the population. The data is therefore grouped in seasons (Table 4).

Although the concentration of elements varied largely between individuals, the levels are within the suggested intake of the EC (EC, 2006). The guidelines for the maximum levels of contaminants in fishery products and fish meat of the EC for mercury (0.5 mg kg^{-1} ww), lead

Table 4
Trace elements and PCBs of European plaice during three different seasons. n = pooled samples constituting of 3 individuals per sample. Results presented as mean values ± SD.

Trace elements	Season September n = 2	December n = 2	April n = 2	
	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	P-value***
Vanadium*	24.0 ± 21.3	6.5 ± 3.3	6.7 ± 1.7	0.580
Chromium*	8.2 ± 3.3	6.3 ± 0.6	8.4 ± 2.1	0.621
Manganese	31.0 ± 2.3	44.8 ± 28.0	44.0 ± 4.3	0.680
Nickel*	2.2 ± 0.4	1.4 ± 0.1	2.4 ± 0.7	0.229
Molybdenum	0.65 ± 0.3[a]	0.65 ± 0.06[a]	1.3 ± 0.02[b]	0.033
Silver*	0.01 ± 0.01	0.1 ± 0.1	0.3 ± 0.08	0.221
Cadmium*	0.16 ± 0.03	0.19 ± 0.1	0.2 ± 0.1	0.939
Cobalt	2.7 ± 1.6	1.8 ± 0.09	3.0 ± 1.2	0.597
Lead*	0.53 ± 0.2 mg kg^{-1}	0.76 ± 0.5 mg kg-1	1.1 ± 0.9 mg kg-1	0.637
Arsenic*	45.3 ± 11.8	51.8 ± 11.2	79.7 ± 49.0	0.544
Iron	1.0 ± 0.3	0.95 ± 0.2	0.82 ± 0.3	0.771
Zinc	3.4 ± 0.02	4.0 ± 0.3	4.3 ± 0.5	0.142
Selenium	0.3 ± 0.09	0.4 ± 0.08	0.5 ± 0.2	0.504
Copper	0.1 ± 0.007	0.1 ± 0.002	0.13 ± 0.03	0.229
Mercury*	0.11 ± 0.1	0.15 ± 0.02	0.09 ± 0.06	0.642
Σ toxic elements	45.4 ± 11.9	51.9 ± 11.2	79.8 ± 49.1	0.545
*toxic elements				
PCBs	September n = 2 ng g^{-1}	December n = 2 ng g^{-1}	April n = 2 ng g^{-1}	P-value***
PCB-3	0.012 ± 0.01	0.123 ± 0.17	<LOD	0.493
PCB-8	<LOD	<LOD	<LOD	/
PCB-28*	<LOD	<LOD	<LOD	/
PCB-52*	0.010 ± 0.01	<LOD	0.015 ± 0.02	0.631
PCB-101*	<LOD	0.147 ± 0.07	<LOD	/
PCB-118**	0.076 ± 0.01	0.141 ± 0.001	0.092 ± 0.065	0.347
PCB-138*	0.180 ± 0.027	0.420 ± 0.154	1.886 ± 2.22	0.455
PCB-153*	0.138 ± 0.02	0.362 ± 0.11	0.563 ± 0.36	0.289
PCB-180*	<LOD	<LOD	0.706 ± 0.99	/
PCB-195	<LOD	<LOD	<LOD	/
PCB-206	<LOD	<LOD	<LOD	/
PCB-209	<LOD	<LOD	<LOD	/
Σ ICES-6*	0.368 ± 0.058	0.698 ± 0.326	0.658 ± 3.55	0.449
Σ DL-PCBs**	0.076 ± 0.01	0.141 ± 0.001	0.092 ± 0.065	0.347
Σ total PCBs	0.416 ± 0.03	1.192 ± 0.15	3.262 ± 3.62	0.467

*ICES-6: non dioxin-like PCBs (NDL-PCBs).
**dioxin-like PCBs (DL-PCBs; Σ of non-ortho + mono-ortho PCBs).
***ANOVA was applied to detect differences in trace elements and PCBs; where significant difference was detected (α < 0.05), a Tukey PostHoc test was applied. Values with different superscript ([a,b]) within a row are significantly different (P < 0.05).

(0.3 mg kg^{-1} ww), and cadmium (0.1 mg kg^{-1} ww) are not exceeded in fish examined in this study (EC, 2006). According to EC (2006) around 90 % of total mercury is present in the form of methylmercury, which is most alarming in fish and seafoods. Methylmercury is the predominant form of inorganic mercury, which can origin from the earth's crust, but also from anthropogenic sources (EFSA, 2012). The EFSA CONTAM Panel sets a TWI of 1.3 μg kg^{-1} body weight (b.w.) for methylmercury, expressed as mercury (EFSA, 2012). Taking the maximum value of 170 μg kg^{-1} total mercury found in fish of December as a basis for calculating the TWI for a person with 60 kg body weight, 459 g European plaice can be consumed per week. This would account for approximately 2.5 portions (180 g portion) of European plaice and is higher than the recommendation of EFSA of 300 g (EFSA, 2014) seafood per week. The analysis of variance showed no significant differences for each element with regards to both locations and seasons ($P > 0.05$). Only the concentration of the essential element molybdenum varied significantly ($P = 0.033$) between the three seasons, being double the concentration in April (1.3 ± 0.02 μg kg^{-1}) compared to September (0.65 ± 0.3 μg kg^{-1}) and December (0.65 ± 0.06 μg kg^{-1}). Afonso et al. (2013) studied different trace elements in five fish, among those megrim (*Lepidorhombus whiffiagonis*) and four spotted megrim (*Lepidorhombus boscii*), two flatfish species with comparable proximate composition to European plaice. Comparable values of Zn with slightly higher values in Fe compared to values found in this study are shown by Afonso et al. (2013). High values of total arsenic were found in all three seasons, with a particular high SD in April. Seafood in general has higher concentrations of total arsenic compared to other food commodities. The greatest part is in the form of organic, non-toxic arsenobetaine, whereas inorganic forms such as arsenite (As + 3) and arsenate (A + 5) are highly toxic and carcinogenic for humans (Sloth, Larsen, & Julshamn, 2005). The CONTAM Panel points out the limited amount of published data on inorganic arsenic levels of seafood and announced that the previously established provisional TWI of 15 μg kg^{-1} can be regarded as too high (EFSA, 2009). Furthermore, EFSA (2009) sets a benchmark dose lower confidence limit ($BMDL_{01}$) of 0.3 to 8 μg kg^{-1}b.w. per day, where BMDL01 explains benchmark dose concentrations for a 95 % lower confidence limit of 1 % extra risk for lung, skin or bladder cancer or skin lesions. Sloth et al. (2005) analysed inorganic arsenic in different marine species, including fish and pointed out that among all samples, the amount of inorganic arsenic constituted to<1 % of the total arsenic content. Expecting a maximum of 1 % inorganic arsenic in fish captured for this study, this would mean a maximum of 1.29 mg kg^{-1} (April) and a minimum of 0.34 mg kg^{-1} (September). Following the recommendation of 8 μg kg^{-1} as an upper $BMDL_{01}$b.w. per day for a person with 60 kg b.w., this would lead to a recommended dietary allowance (RDA) of 372 g European plaice per day considering the maximum values of inorganic Arsenic in April. This leads to a PTWI of at least 2.6 kg European plaice for a safe consumption.

The analysis of several PCBs included both non dioxin-like PCBs (NDL-PCBs) as well as one dioxin-like PCB congener (DL-PCB; PCB-118). Several concentrations of the targeted PCBs were lower than the detection limit. This indicates a non-significant concentration, hence no major exposure to these PCB congeners when consuming this fish. According to the regulation of the European Commission (EC, 2011), present results of NDL-PCBs do not exceed the recommended limits of 75 ng g^{-1} wet weight for fish and fishery products. Furthermore, the total amount of detected PCBs in the samples is at a low level, being around 0.4, 1.19 and 3.26 ng g^{-1}, respectively. No statistical significance between the three seasons could be found. However large differences in standard deviations indicate large discrepancies in the pooled samples, hence imply larger variations between individuals. Unexpectedly, the concentration of PCB-118 (0.14–0.07 ng g^{-1}), being a DL-PCB exceeds the recommended upper limit 6.5 pg g^{-1} (EC, 2011) in all three seasons. Particularly high concentrations (0.141 ± 0.001 ng g^{-1}) were found in pooled samples of December. Previously, foods with <1 % fat have been excluded from regulations on the maximum level of dioxins and DL-PCBs (EC, 2011), as these chemicals tend to accumulate in the adipocytes of organisms. The accumulation of DL-PCBs could be a result of the higher lipid content compared to the other two seasons.

Concerning the analysis of toxic trace elements and PCBs, values exceeding the recommended levels for DL-PCBs (PCB-118) were obtained, while values corresponding to e.g., lead, total mercury or cadmium can be considered within the acceptable limit. The risk of possibly elevated levels of DL-PCBs can be opposed to the positive nutritional aspects, including a good distribution of essential amino acids as well as n3 fatty acids of European plaice. To our knowledge, so far, no risk–benefit assessment on increasing the consumption of European plaice has been carried out.

4. Conclusion

In this study, chemical analyses and screening of toxic elements and PCBs in European plaice throughout three seasons showed predominantly positive results to promote the consumption of the species in all three seasons. The results indicate stable total essential amino acid distributions in all three investigated seasons, but a variance in PUFA content, with higher amounts of PUFA in fish caught in September. Changes in the total lipid content and lipid composition imply higher lipid storage during December and more membrane lipids in fish caught in April. Values on toxic trace elements revealed no harmful elevated values and the overall PCB content in fish was below the maximum accepted limit for all three seasons. Nevertheless, elevated levels of DL-PCBs (PCB-118) were found in all three seasons, with highest values in December which must be considered. Moreover, more data on inorganic arsenic on fish must be collected to set up a concentration limit for European plaice as well as fish and fishery products in general. Future work should focus on the assessment of possible negative and beneficial effects that come along with an increased consumption of European plaice in Norway.

CRediT authorship contribution statement

Sophie Kendler: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Dionysios Tsoukalas:** Investigation, Conceptualization. **Anita Nordeng Jakobsen:** Conceptualization, Methodology, Supervision. **Junjie Zhang:** Formal analysis, Methodology, Investigation. **Alexandros G. Asimakopoulos:** Resources, Methodology. **Jørgen Lerfall:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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