Fatty acid analyses reveal differences in feeding ecology of North Sea squids that overlap in time and space

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ARTICLE INFO

ABSTRACT

Climate-induced changes in marine ecosystems have been documented worldwide. As one of the main consequences, a shift in the distribution of species is observable in many marine areas, resulting in the formation of new species communities and new interactions. In the North Sea, the squid community has changed considerably over the last 100 years. Some species have disapeared while new species have established and are now living in coexistence in a new community. Although squids are considered to be predators that feed rather non-selectively, we aimed to answer the question of whether their diet differs nevertheless. Therefore, we analysed the fatty acids of three squid species whose distribution substantially overlaps. We were able to recognise a dependence between the size of the squid and the composition of fatty acids and are able to demonstrate the already known ontogenetic shift in food composition on the basis of fatty acid composition. Furthermore, we illustrate that the fatty acid composition differs significantly between squid species, which points to different prey of the analysed squid species and which may be one reason for their successful coexistence.

1. Introduction

Cephalopods play an important role in the marine ecosystems. On the one hand, they serve as food for numerous predators such as large predatory fish, marine mammals and seabirds; on the other hand, larger cephalopods can exert a strong influence in the food web as top predators (Nixon, 1987; Clarke, 1996; Rodhouse and Nigmatullin, 1996; Boyle and Rodhouse, 2005; Gasalla et al., 2010). In general, cephalopods are predators and are able to eat anything they can get their arms around and overpower (e.g., Budelmann, 1996; Hanlon and Messenger, 2018; Villaneuva et al., 2017). In many cases, small cephalopods tend to eat crustaceans, polychaetes and chaetognaths, but the proportion of fish and cephalopod prey become more important with increasing size of the cephalopods (e.g., Collins et al., 1994; Pierce et al., 1994; Wangvoralak et al., 2011; Oesterwind and Piatkowski, 2023), in some areas the increase of cephalopod abundances has resulted in a community shift towards squid-dominated food webs (Caddy and Rodhouse, 1998; Balguerías et al., 2000; Hunsicker et al., 2010; Doubleday et al., 2016).

In certain areas, cephalopod abundance and biomass are increasing (Doubleday et al., 2016), whereas there are also some areas where cephalopod abundance and biomass seem to decrease (Ospina-Alvarez et al., 2021). Where biomasses and abundances are increasing, they often increase substantially as it is the case for the North Sea where also new species have established (Oesterwind et al., 2022). This raises the question whether prey composition differs between species so much that a coexistence between the species is possible. Lin et al. (2020) suggested that a spatial segregation is likely the major mechanism leading to the coexistence of four loliginid species in costal waters of the northern South China Sea, while Valls et al. (2015) identified a bathymetric segregation between *L. forbesii* and *L. vulgaris* in the Mediterrenean Sea. In contrast, North Sea squids show strong spatial and seasonal overlap (De Heij and Baayen, 2005; Oesterwind et al., 2010) and the bathymetry of the North Sea makes a bathymetric segregation very unlikely. Therefore, we investigate whether also other mechanisms are relevant for the coexistence of different squid species within the same ecosystem. The North Sea, a marginal sea of the Northeast Atlantic, is a high productivity shelf sea. Among other cephalopod species, three Loliginidae (*Loligo forbesii, Loligo vulgaris, Alloteuthis media* – the latter has formerly

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been reported as *A. subulata*) and three Ommastrephidae inhabit the North Sea year around (*Todaropsis eblanae, Illex coindetii*) or at least seasonally (*Todarodes sagittatus*) (Goud et al., 2019; Oesterwind et al., 2022; Sheerin et al., 2023), with highest abundances for *L. forbesii* and *A. media* (reported as *A. subulata* in: De Heij and Baayen, 2005; Oesterwind et al., 2010) and lowest abundances for *T. sagittatus*.

Although some feeding studies from the North Sea and its adjustance waters have already been published (Collins et al., 1994; Pierce et al., 1994; Wangvoralak et al., 2011; Oesterwind and Piatkowski, 2023), the general importance of many cephalopod species as predators is still not fully understood (Lishchenko et al., 2021; Bobowski et al., 2023). Studies of the trophic role of North Sea cephalopods are still relatively rare, and several existing studies have been published >25 years ago (Collins et al., 1994; Pierce et al., 1994; Collins and Pierce, 1996; Pierce and Santos, 1996) and may partly no longer be representative of the current food web (Wangvoralak et al., 2011) a rapidly changing environment. Furthermore, in the North Sea, diet analysis of cephalopods have to our knowledge so far only been performed via visual stomach content analysis (e.g., Wangvoralak et al., 2011; Oesterwind and Piatkowski, 2023). Such microscopical observations have the advantage that hard structures such as fish otoliths and vertebrae, cephalopod statoliths and cephalopod beaks can be determined down to species level, and that even the original prey size and body weight can be calculated via regressions. However, constrained by their anatomy, cephalopods need to break down their food into small pieces, and it is observed that some hard parts, for example the head of medium-sized and larger fish, may not be ingested at all (Wallace et al., 1980; Porteiro et al., 1990; Rodhouse and Nigmatullin, 1996). In addition, soft parts are difficult to identify and are digested quickly, consequently squid stomachs are often empty. In such cases, visual stomach content analysis will not provide any valuable information. Furthermore, stomach content analyses in general provide only a snapshot of the prey spectrum, which can also lead to a biased interpretation.

The results, of visual stomach content analysis, assume that North Sea cephalopods are, similar to most cephalopods, opportunistic predators and only small differences are observable between the species (Pierce et al., 1994; Oesterwind and Piatkowski, 2023), whether these small differences are permanent or biased by the possible snapshot provided by the visual stomach content analysis is unknown.

For the investigation of the long-term, or characteristic, feeding habits of cephalopods, alternative methods appear more suitable than visual stomach content analysis, and in particular, this is the case for the analysis of fatty acids in the animals' tissues. Fatty acids can be used to identify trophic relationships and energy transport through the food web (e.g., Sargent, 1978) and have often been successfully applied in aquatic ecosystems (Lee et al., 2006; Schukat et al., 2013; Peters et al., 2015). A major advantage of measuring fatty acids is, that they provide information on the typical or average diet consumed over a period of time, even when the stomach is empty at the time of the analysis (Iverson et al., 2004).

We here investigated the fatty acid composition in three squid species from the North Sea, namely the ommastrephids *Illex coindetii* and *Todaropsis eblanae* as well as the loliginid *Loligo forbesii*. All three species are important commercially used squids in the Northeast Atlantic and in the Mediterreanean Sea, and their biomasses increased in the North Sea within the last decades. At the end of the 19th and beginning of the 20th century, *L. forbesii* was just a regular guest for spawning in the North Sea, but has since become a permanent inhabitant with two spawning stocks

(Pierce et al., 2005; Oesterwind et al., 2022, Oesterwind et al., 2010; Bobowski et al., 2023). In winter *L. forbesii* occurs more frequently in the northern North Sea while in summer the species is present throughout the entire North Sea (Oesterwind et al., 2010). About 100 years ago, *T. eblanae* and *I. coindetii* were only accidental visitors in the North Sea, since then both species have extended their regular distribution range from the Irish Sea to the North Sea. While *T. eblanae* most likely already permanently inhabited the North Sea in the middle of the 20th century and formed an independent stock (Oesterwind et al., 2022), *I. coindetii* only became established at the beginning of the 21st century (Oesterwind et al., 2020). Currently both species are distributed in the northern and central parts of the North Sea with a slightly more southern distribution for *T. eblanae* in summer with assumed northern spawning grounds while *I. coindetii* seems to spawn all over the North Sea (Oesterwind et al., 2015; Barrett et al., 2021).

In combination with increasing stock size, all three squids can have a significant impact on the food web. Furthermore, due to their large size they can also exert top-down pressure on the fish fauna (Wangvoralak et al., 2011; Oesterwind and Piatkowski, 2023). Because the distribution of all three species overlaps substantially it is therefore an excellent opportunity to investigate whether they show any differences in feeding ecology although they are known as opportunistic carnivores. We therefore hypothesis that the three species should show a similar feeding ecology, and thus would not be expected to differ significantly in their fatty acid composition. In an initial step, we describe the average lipid content and fatty acid composition of the three species, before testing the hypothesis that fatty acid composition and lipid content would not be species-specific. Furthermore, since previous stomach content analyses suggested an onthogenetic shift in diet (Mangold, 1983; Lipinski, 1987; Pierce et al., 1994), we use the example of *L. forbesii* in order to test whether the observerd fatty acid composition depends on the animals' size, as represented by the dorsal mantle length (DML). If our results show any dependencies between length and the content of the fatty acids and lipids, we will only compare similar length classes of cephalopods.

2. Materials and methods

2.1. Field sampling

Cephalopods were caught during two fisheries research cruises in the North Sea, specifically the 'Walther Herwig III' cruises WH 443 and WH 464 in quarter 1 (Q1, January/February) of 2021 and 2023, respectively. Both cruises were conducted within the International Bottom Trawl Survey (IBTS, ICES, 2020) which is coordinated by the International Council for the Exploration of the Sea (ICES). Catches were obtained using the standard gear of the IBTS, a GOV (Grand Ouverture Vertical) otter board bottom trawl (ICES, 2020). Sampling stations were located in the northern North Sea (Fig. 1).

In total 92 samples comprising three different species were analysed: *Illex coindetii* (n = 29), *Loligo forbesii* (n = 47), and *Todarodes eblanae* (n = 16; Table 1). The cephalopods were frozen aboard and stored at −20 °C until dissection in the laboratory ashore. When thawed, individual lengths and weights of the specimens were recorded, and tissue samples for the lipid analyses transferred to Eppendorf vials.
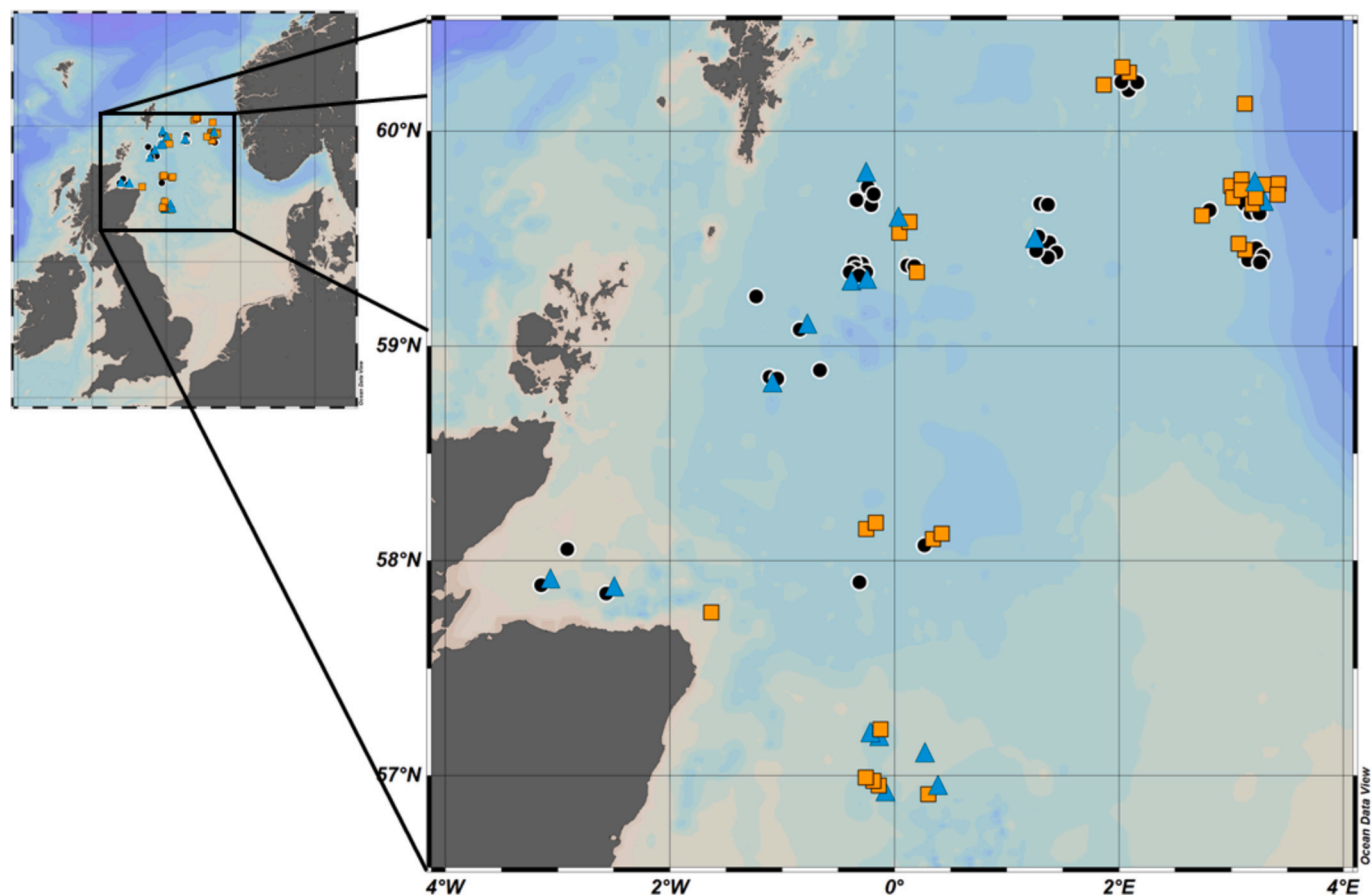
Fig. 1. Sampling positions for the three cephalopod species analysed in this study, orange squares: *Illex coindetii* (*n* = 29); black circles: *Loligo forbesii* (*n* = 47); light blue triangles: *Todaropsis eblanae* (*n* = 16). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Numbers of samples analysed per squid species and year.

∑	Illex coindetii	Loligo forbesii	Todaropsis eblanae
Q1 2021	19	37	6
Q1 2023	10	10	10
total	29	47	16

2.2. Lipid extraction and fatty acid analysis

Tissues of squid were freeze dried for 16 h at 0.3 mbar. Lipids were extracted from the dry mass using the method described by Smedes (1999). Total lipids were dissolved in chloroform and stored at −20 °C under the absence of light until further analysis. For fatty acid methyl esther (FAME) analysis, methyl esters were prepared by two step transesterifications (IUPAC, 2024). The lipid solution containing 5 to 25 mg lipid was placed in a reaction vessel and the solvent was removed in the nitrogen steam. After the addition of sodium methoxide solution (0.2 mol/l; 2 ml) the sample was stirred for 30 min at 100 °C under nitrogen atmosphere until the solution became clear. The sample was cooled to room temperature before methanol (6.5 ml), methanolic phenolphthalein solution (2 drops) and methanolic sulfuric acid (1 mol/l;0.65 ml) were added and the solution was stirred again for 30 min at 100 °C. Following cooling to room temperature again, potassium chloride solution (saturated; 4 ml) and 2,2,4-trimethylpentane (1.5 ml) were added and the mixture was shaken vigorously. After phase separation the reaction vessel was filled with potassium chloride solution until the liquid level reached the bottle neck. The organic phase containing the FAME was removed and dried over sodium sulfate. The methyl ester solution was filled into a nitrogen rinsed vile and additionally a 1:10 dilution in 2,2,4-trimethylpentane was prepared in another nitrogen rinsed vile. Both solutions were stored at −20 °C until gaschromatographic analysis (maximum of one day).

Methyl esters of 37 individual fatty acids were analysed using a GC-FID (Agilent 8890) equipped with a capillary column (DB-fatwax UI; 30 m × 0.25 mm × 0.25 μm). Following conditions for the analysis were used: Split injection: Split ratio: 1:50; Inlet temperature: 250 °C; carrier gas: Helium, gas-flow: 1.2 ml/min (constant flow); Oven program: 180 °C for 2 min; heating to 210 °C with a rate of 2 °C per minute; 210 °C for 35 min; FID temperature: 280 °C; hydrogen flow: 40 ml/min. Identification of individual FAME was achieved by comparison of retention times with 37-component FAME mixture, PUFA No 1 and PUFA No 3 by Supelco (purchased from Sigma Aldrich). For quality assurance purposes the developed method was tested with standard reference material for fish oil (NIST 7250–1 to −3). The precision of the whole method is at 10% related to the absolute values (in mg) of the measured FAs. Limits of detection (LOD) ranged from 0.04% (0.011 mg; C17:0) to 0.63% (0.037 mg; C16:0), whereas limits of quantification (LOQ) varied between 0.13% (0.032 mg; C17:0) and 1.90% (0.110 mg; C16:0) for all FAs under investigation. Individual results as well as means below LOQ were omitted from evaluation (C15:0 and C16:1(n7) for *I. coindetti*). From the 37 investigated FAs only 12 (10 for *I. coindetii*) were on average above the LOQ and thus were included in further analyses. All values are presented as percentages of the sum of the 12 (10 in case of *I. coindetii*) fatty acids.

2.3. Visualization & Statistics

The sampling map was created in Ocean Data View version 5.6.5 (Schlitzer, 2023). All statistical analyses were carried out using Statistica

Version 12.5 (Statsoft Europe, Hamburg Germany). For revealing individual fatty acids contributing to a possible species-specific difference in fatty acid composition, univariate ANOVA in combination with a Fisher LSD post-hoc test was used, both with 95% significance. A principal component analysis (PCA) was performed using varimax rotation with a reduced set of fatty acids selected by significant results ($p < 0.005$) in the univariate ANOVA. This was done to reduce numbers of (partly correlated) variables and to enhance the level of variance explained by PCA at the same time. PCA was chosen to reveal and display relations in the data set. A linear discriminant analysis was conducted using Posteriori probabilities. It was chosen to investigate if the species can be truly predicted by fatty acids.

3. Results

3.1. Lipid content and fatty acid composition

Mean lipid contents were similar in the two cephalopod species *L. forbesii* and *T. eblanae*, amounting to 8.34% and 8.76% of the dry weight, respectively. In contrast, the lipid content of *I. coindetii* exhibited the slightly lower mean value of 6.03% (Table 2). Individual lipid contents showed a high variability (Fig. 2). Overall, poly-unsaturated FAs dominated and reached between 56% and 60% in all three squid species. Within PUFAs, the sum of omega-3 FA ranged from 55.07% in *L. forbesii* to 56.91% in *T. eblanae* and 59.05% in *I. coindetii* (Table 2). Omega-6 FA amounted to much lower fractions, which were similar between species, ranging from 1.03% (*I. coindetii*) to 1.28% (*T. eblanae*; Table 2). Saturated FAs made up about a third of the FAs and reached similar proportions in all three species. Mono-unsaturated FAs, while again similar in quantity between species, were most abundant in *L. forbesii* (10.65%) and lowest in *T. eblanae* (7.88%, Table 2).

At the same time, lengths of the squids - given as DML - cover a wide range from about 50 to 340 mm (Fig. 2). Mean lengths varyied considerably between the species ranging from 68 ± 13 mm in *T. eblanae* to 181 ± 59 mm DML in *L. forbesii*, respectively (Table 2). For *L. fobesii*, where the investigated specimens covered a wide range of body sizes, a non-significant positive correlation ($p = 0.227$; $r^2 = 0.032$) between DML and lipid content could be observed (Fig. 2). Furthermore, a non-significant negative correlation between DML and lipid content exist for *T. eblanae* ($p = 0.019$; $r^2 = 0.333$) and *I. coindetii* ($p = 0.538$; $r^2 = 0.014$).

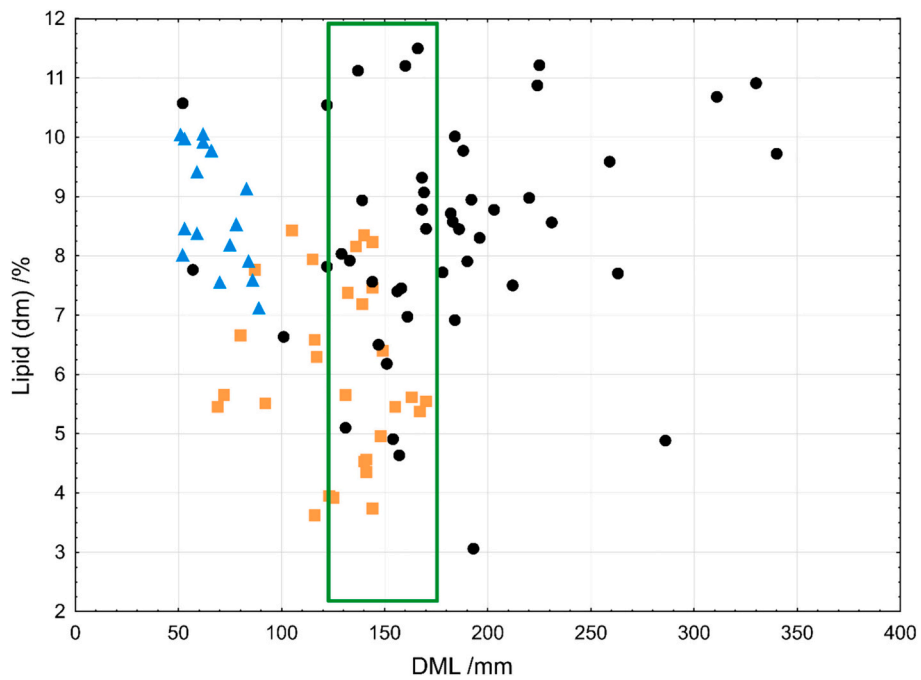### 3.2. Influence of individual length on fatty acid composition

To reveal the variation of fatty acid composition within each of the species, two size classes with the largest and the smallest individuals of each species were selected and their fatty acid composition were compared (Supplement Table S1). A clear influence of individual length on FA composition was seen in all three species under investigation. The occurrence of various FA showed significant differences with size: this was seen specifically in five FA for *L. forbesii* (C14:0, C18:0, C18:1, C20:1, C20:5), in six FA for *T. eblanae* (C14:0, C15:0, C16:0, C16:1, C18:1, C22:6) and in three for *I. coindetii* (C18:0, C20:1, C20:4). In more detail, for *L. forbesii* we observed significant decreases for both, C18:1 (n7) and C20:5 (n3) with size, and increases for C14:0, C18:0 and C20:1 (n9). C22:6(n3) was also increasing, although this trend was not statistically significant. Furthermore, for *L. forbesii* and *T. eblanae* also the lipid contents were signifivantly different between the group of smallest and largest individuals.

### 3.3. Species-specific differences in fatty acid composition

Due to the size depending FA composition, we chose a common length range for a comparison between species in order to exclude the size effect. *T. eblanae* was excluded from the species comparison, because only smaller individuals of *T. eblanae* were available. In an iterative process the length range from 120 to 170 mm DML was selected for the comparison between *I. coindetii* and *L. forbesii* (Fig. 2, Supplement Table S2), providing a sufficient number of individuals ($n = 41$). ANOVA with the FA percentages of individual samples revealed significant differences between *I. coindetii* and *L. forbesii* for the following six FAs: C14:0, C18:0, C18:1(n7), C18:1(n9), C20:5(n3), and C22:6(n3) ($p < 0.005$; Fig. 3). Furthermore, C15:0 and C16:1(n7) might also have been promising candidates to discriminate between both species. However, because these FAs were below LOQ for many individuals of *I. coindetii* but above LOQ for *L. forbesii*, the observed difference could not be tested for significance.

With the six selected FAs a PCA was conducted on the entire data set for *I. coindetii* and *L. forbesii* explaining 79.75% of the variance with the first two factors (Fig. 4). Factor 1 explains 59.20% of the variance and refers mainly to C18:1(n9) and inversely to C22:6(n3). Factor 2 explains 20.54% of the total variance and is dominated by C18:0 and C18:1(n7). However, the secondary variable "lipid" is related to factor 1, because it

Table 2
Dorsal mantle length (DML), lipid content related to wet weight (ww) or to dry matter (dm) respectively, as well as fatty acid composition (percentages of their sum) investigated in: *I. coindetii*, *L. forbesii* and *T. eblanae*. Presented are numbers or mean values and standard deviation in brackets. n.d.: not determined. Sums n3: n3-fatty acids; n6: n6- fatty acids, SFA: Saturated fatty acids, MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids.

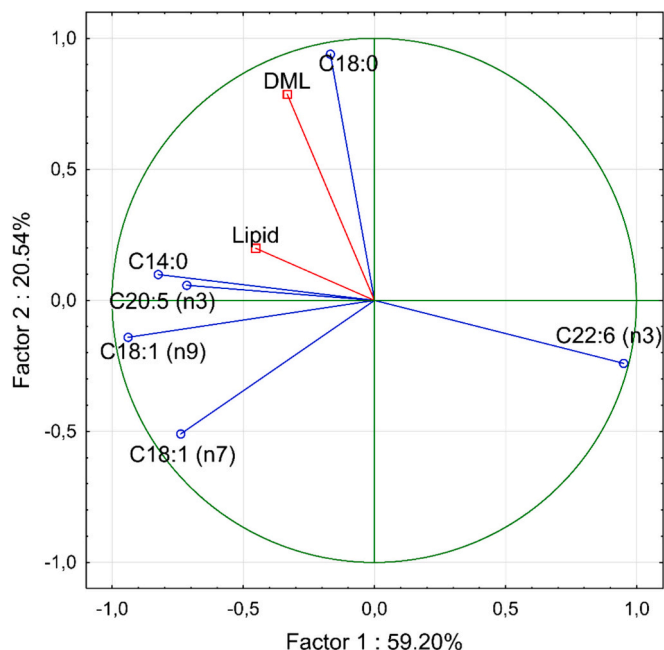| Species (n)	*Illex coindetii* (29)	*Loligo forbesii* (47)	*Todaropsis eblanae* (16)	All (92)
DML [mm]	128 ($\pm$27)	181 ($\pm$59)	68 ($\pm$13)	145 ($\pm$62)
Lipid [ww %]	1.26 ($\pm$0.25)	1.71 ($\pm$0.36)	1.59 ($\pm$0.23)	1.55 ($\pm$0.37)
Lipid [dm %]	6.03 ($\pm$1.51)	8.34 ($\pm$1.92)	8.76 ($\pm$1.00)	7.69 ($\pm$2.01)
C14:0	2.18 ($\pm$0.26)	3.38 ($\pm$0.60)	3.48 ($\pm$0.53)	3.02 ($\pm$0.76)
C15:0	n.d.	0.58 ($\pm$0.10)	0.64 ($\pm$0.08)	0.41 ($\pm$0.29)
C16:0	25.71 ($\pm$0.95)	25.12 ($\pm$0.89)	25.72 ($\pm$0.12)	25.41 ($\pm$0.99)
C16:1 (n7)	n.d.	0.97 ($\pm$0.24)	0.56 ($\pm$0.08)	0.59 ($\pm$0.46)
C17:0	0.65 ($\pm$0.14)	0.59 ($\pm$0.14)	0.68 ($\pm$0.08)	0.63 ($\pm$0.13)
C18:0	3.18 ($\pm$0.32)	3.54 ($\pm$0.59)	3.41 ($\pm$0,55)	3.40 ($\pm$0,53)
C18:1 (n9)	2.67 ($\pm$0.32)	3.97 ($\pm$0.42)	2.31 ($\pm$0.34)	3.27 ($\pm$0.82)
C18:1 (n7)	1.41 ($\pm$0.20)	1.73 ($\pm$0.24)	1.14 ($\pm$0.16)	1.52 ($\pm$0.31)
C20:1 (n9)	4.12 ($\pm$0.48)	3.99 ($\pm$0.57)	3.87 ($\pm$0.54)	4.01 ($\pm$0.54)
C20:4 (n6)	1.03 ($\pm$0.16)	1.07 ($\pm$0.22)	1.28 ($\pm$0.39)	1.09 ($\pm$0.26)
C20:5 (n3)	14.68 ($\pm$2.12)	16.88 ($\pm$0.68)	14.98 ($\pm$3.61)	15.86 ($\pm$2.21)
C22:6 (n3)	44.37 ($\pm$1.46)	38.19 ($\pm$1.29)	41.93 ($\pm$2.65)	40.79 ($\pm$3.24)
Sum n3	59.05 ($\pm$1.16)	55.07 ($\pm$1.23)	56.91 ($\pm$2.29)	48.20 ($\pm$10.06)
Sum n6	1.03 ($\pm$0.16)	1.07 ($\pm$0.22)	1.28 ($\pm$0.39)	1.09 ($\pm$0.26)
Sum SFA	31.72 ($\pm$0.93)	33.21 ($\pm$1.25)	33.29 ($\pm$1.73)	32.76 ($\pm$1.43)
Sum MUFA	8.20 ($\pm$0.57)	10.65 ($\pm$1.03)	7.88 ($\pm$0.85)	9.40 ($\pm$1.56)
Sum PUFA	60.08 ($\pm$1.09)	56.13 ($\pm$1.26)	58.19 ($\pm$2.46)	57.74 ($\pm$2.30)

Fig. 2. Individual samples displayed by their lipid content [%] and dorsal mantle length (DML). Orange squares: *Illex coindetii*; black circles: *Loligo forbesii*; light blue triangles: *Todaropsis eblanae*; green box: Size range from 120 to 170 mm DML for inter-species comparison. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
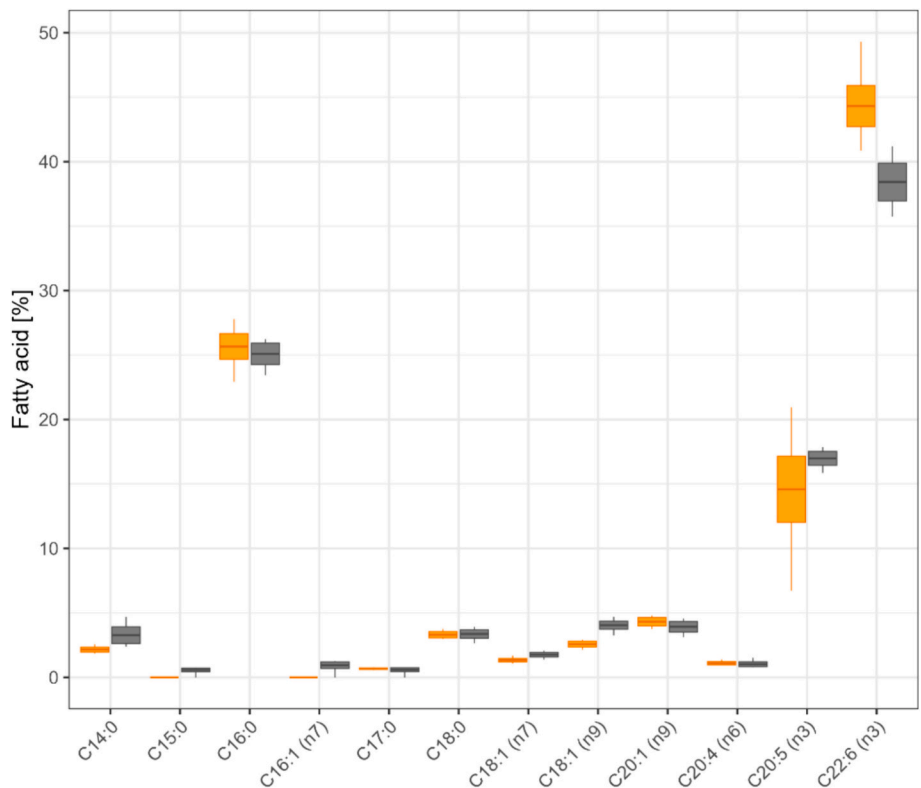
Fig. 3. Fatty acid composition [%] of squid in size range 120–170 mm DML. Given are mean values +/− standard deviations (box), as well as minima and maxima (whiskers). Orange: *Illex coindetii*; black: *Loligo forbesii*.

is displayed closer to the horizontal axis than to the vertical one in Fig. 4. The factor projection of the samples (Fig. 5) showed a good but not perfect separation of the two species via FA composition by the first two PCA factors.

The above-mentioned six selected FAs were subjected to a linear discriminant analysis leading to a true classification of the two species with a classification error of zero out of 76 samples. Comparable results could be obtained by using oleic acid, C18:1(n9), and docosahexaenoic

Fig. 4. Principal component analysis (PCA) of percentual fatty acid composition in two squid species *Illex coindetii* and *Loligo forbesii*. Secondary variables: Lipid = Lipid content [%] related to tissue dry matter; DML = dorsal mantle length [mm]; blue: PCA variables; red: additional variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acid (DHA), C22:6(n3), only. The result of discriminant analysis supports in general the outcomes of ANOVA and PCA as described above. However, also other FA combinations may lead to good classifications too, but were not further investigated.

4. Discussion

4.1. Lipid content

Hosting a protein-based metabolism, cephalopod tissue is mostly composed of proteins (~20% of wet body weight), with only a very small percentage of lipids, ranging from $3.39 \pm 0.48\%$ DW in mantle tissue of *Ophitoteuthis* sp. and $6.27 \pm 0.78\%$ DW in mantle tissue of *I. condetii* (Rosa et al., 2005). This is very close to the results of the present study, where mean lipid contents between $6.03 \pm 1.51\%$ and $8.76 \pm 1.00\%$ DW were measured (Table 2). The total lipid contents already indicate some inter-species differences with *I. coindetii* having the lowest and *L. forbesii* the highest values. As it seems characteristic for squids (Ozogul et al., 2008), the fatty acid patterns for all species investigated here are dominated by docosahexaenoic acid DHA C22:6 (n3), eicosapentaenoic acid EPA C20:5(n3), and palmitic acid C16:0, which together represented >80% of the total fatty acids.

Interestingly, the dry matter lipid content of *L. forbesii* and *T. ebalanae* were more similar between these two species than compared to *I. coindetii*, even though mean DML differed more between *L. forbesii* and *T. eblanae*, than between *L. forbesii* and *I. coindetii*. Indeed, Rasero et al. (1996) indicate that *I. coindetii* has a broader and more pelagic diet compared to *T. eblanae*. Therefore, *T. eblanae* diet composition might be accordingly more comparable to the demersal squid *L. forbesii*, which is reflected in our results.

Rosa et al. (2005) were able to separate different life strategies of cephalopods by comparing the biochemical composition of the species, so that their results might explain the similarity between *L. forbesii* and *T. eblanae*. Lordan (2001) mentioned that, *T. eblanae's* morphometrics and migratory behaviour seems to be more similar to neretic loliginids like *L. forbesii* than to sympatric ommastrephid species like *I. coindetii*, which indicate a similar life strategy between *L. forbesii* and *T. eblanae*. Yet, Lordan's observations stem from Irish waters and it remains speculative whether they are transferable to the shallow North Sea ecosystem.
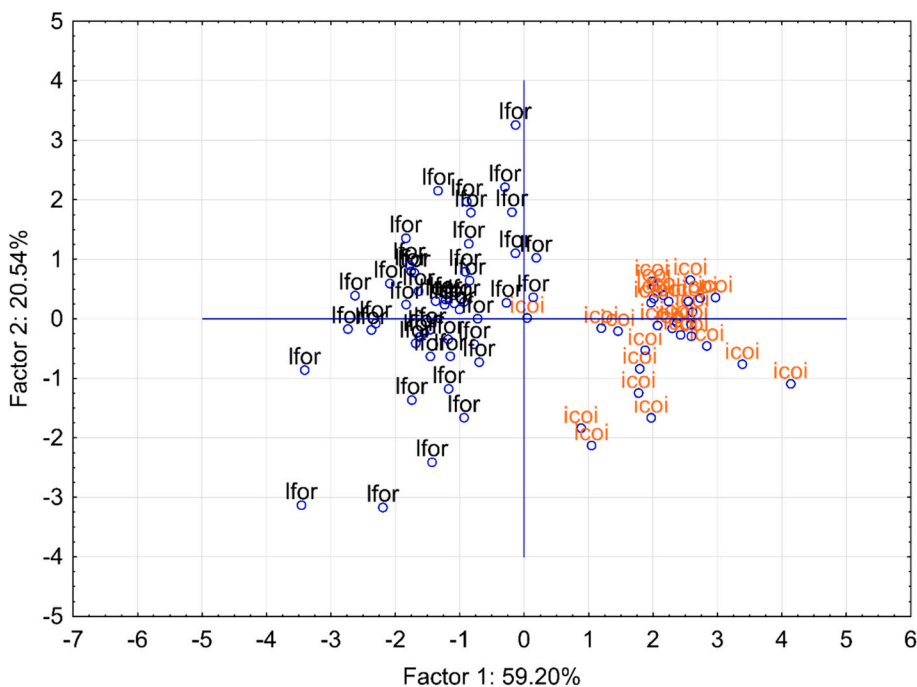
Fig. 5. Factor projection in principal component analysis of percentual fatty acid composition in two squid species *Illex coindetii* (icoi) and *Loligo forbesii* (lfor). Data points are marked by their species.

4.2. Size-dependent FA composition

Our results clearly illustrate that the fatty acid composition of the same species depends strongly on DML. This supports earlier studies describing an ontogenetic shift in squid diets based on visual stomach content analysis (e.g. Mangold, 1983; Lipinski, 1987; Oesterwind and Piatkowski, 2023). Different FAs showed significant differences between small and large individuals. For *L. forbesii* we observed a significant decrease in the percentages of C20:5(n3) for increasing DML. The resulting reduction in the EPA/DHA ratio, which may be used as a carnivority index (Dalsgaard et al., 2003; Meyer et al., 2019), suggest an ontogenetic shift towards a higher trophic level in *L. forbesii*. In addition, we detected a significant increase in C20:1(n9) with body size, which is used as a biomarker for some copepod species (Kattner and Hagen, 1995; Iverson, 2009; Schukat et al., 2013). Since larger squids are not known for feeding on copepods it might be an indication for an expanded consumption of copepod consumers like fish.

4.3. Species-specific differences in FA composition

We were able to show that similarly sized, coexisting *L. forbesii* and *I. coindetii* can be differentiated by their FA composition. Major differences between the two species were observed for the contents of EPA and DHA.

Between the two squid species *I. coindetii* and *L. forbesii*, we observed the greatest differences in FA composition relating to the concentrations of C18:1(n9) – which was more abundant in *L. forbesii* - and inversely to DHA C22:6(n3), which dominated in *I. coindetii*. The monounsaturated C18:1(n9), oleic acid, occurs in various animal and plant fats or oils, and is the most common fatty acid in nature. Elevated levels of DHA have previously been shown to occur in migratory marine fishes such as tuna (Saito et al., 2005) and mackerel (Osako et al., 2006).

Since *I. coindetii* tissues contain less EPA and more DHA, the carnivorous index for *I. coindetii* (0.33) is lower than for *L. forbesii* (0.44). This suggests a more fish-based nutrition and a higher trophic level for *L. forbesii* at comparable body size. Contrary, *I. coindetii* shows higher proportions of C20:1n9, which can be an indication of an increased fish consumption as mentioned above. A comparison between *I. coindetii* and *T. eblanae* show similar changes in the shares of the fatty acids, even though not all differences are statistically significant.

Not all trends in the fatty acid patterns for all investigated species are consistent, and indicates the opportunistic feeding behaviour. But our results clearly illustrate that the fatty acid composition of the same species depends strongly on DML and therefore indicate an ontogenetic shift as already described by visual stomach content analysis (Oesterwind and Piatkowski, 2023).

4.4. Limitations of the study

FA studies of digestive gland, that were not part of the study could possibly give a clearer insight to confirm our hypothesis. Several studies have shown that the lipid content in the midgut gland is much higher than in the muscle tissue of the mantle (Phillips et al., 2002; Rosa et al., 2005; Berge and Barnathan, 2005).

The number of individuals available for this study prevented a full multivariate analysis, which would include individuals of all size classes for each of the species. Therefore, we can only conclude in the size effect for the *L. forbesii*, and could only test the species-specificity in the comparison between *L. forbesii* and *I. coindetii*.

Even if we were able to illustrate differences in fatty acid composition between species, an exact interpretation and thus precise conclusions about the food is difficult to achieve. A combination with additional methods such as isotope measurements, genetic and visual stomach content analyses are necessary for a more precise interpretation of the food composition.

Even though all the individuals analysed were caught in the area of the northern North Sea and some were fished at the same stations at the same time, it cannot be completely ruled out that the results obtained are due to differences in existing small-scale separation, as it is already assumed to be the case on a larger scale (Oesterwind and Piatkowski, 2023), and therefore cannot be attributed to selectivity or even preferences. Therefore, selectivity and preference studies have to be performed.

5. Conclusions

The prey composition differs somewhat between squid species, thus forming a possible coexistence, but a clear spatial and/or seasonal separation as described in other studies (e.g. Valls et al., 2015; Lin et al., 2020) is unlikely. Maybe a clear differentiation in feeding behaviour and thus prey composition between North Sea cephalopod species is not necessary, if nutrient resources are not limited in such a high productive area like the North Sea. Whereas former studies (e.g. Oesterwind and Piatkowski, 2023) were not able to compare the feeding ecology of *L. forbesii* and *I. coindetii* due to the low number of *I. coindetii* stomachs in their study, our present study supports the assumption that at least *I. coindetii* and *L. forbesii*, even while of the same size, feed somewhat differently. However, whether prey selectivity or a small-scale separation through a more demersal or pelagic behaviour is the reason for the differences, cannot be identified in our study.

However, the observed changes in the fatty acid patterns with regard to the sizes of the individuals have to be considered when different species are compared.

Further research involving both visual and genetic stomach content analysis, as well as other biomarker like stable isotopes, in feeding ecology of cephalopd should be conducted for a better interpretation of our results.

CRediT authorship contribution statement

Fabian Schäfer: Writing – review & editing, Writing – original draft, Investigation. **Daniel Oesterwind:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Anne F. Sell:** Writing – review & editing, Conceptualization. **Ulrike Kammann:** Writing – review & editing, Visualization, Resources, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

There is no conflict of interest of any co-author.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fooweb.2024.e00355.

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