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Linking lipid dynamics with the reproductive cycle in Baltic cod *Gadus morhua*

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ABSTRACT: This study describes lipid composition and antioxidants of Baltic cod *Gadus morhua* L. during the reproductive cycle, and investigates whether they reflect its dominant prey and whether levels of fatty acids important for reproductive performance were low. Reasons for a shift in peak spawning time of Baltic cod from spring/early summer to midsummer since the early 1990s remain unresolved and may partly be diet related. This study demonstrated that a substantial amount of lipid was invested in cod ovarian development, and that lipid composition varied substantially with the reproductive cycle. Selective retention of the essential fatty acids docosahexaenoic acid (DHA) and arachidonic acid (ARA) in ovaries during maturation was evident, but despite mobilization from the liver, ARA levels were low in ovaries during late maturation and spawning. Astaxanthin and α-tocopherol accumulated in cod ovaries and decreased in late maturing and spawning fish, most likely due to their antioxidant protection activity. The fatty acid composition of cod liver reflected its clupeid prey. The ratio of 18:1n-9 to DHA was almost twice as high in sprat as in herring and indicated the ratio of sprat and herring in cod diet, while the level of 16:1n-7 and astaxanthin indicated the presence of the isopod *Saduria entomon* in cod diet. It is likely that food web alterations in the Baltic ecosystem related to environmental and hydrographic changes caused a decrease in ARA availability. Low ARA content coincides with cod ovarian development in the central Baltic Sea, and may be associated with the delay in spawning and affect egg and larval survival; however, this needs further verification in experimental studies.

KEY WORDS: Arachidonic acid · Astaxanthin · Baltic Sea · Fatty acid composition · Maturation · Predator–prey relationship · Reproduction · Trophic interaction

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INTRODUCTION

A remarkable shift in the peak spawning time of cod in the central Baltic Sea (ICES subdivisions 25 to 32) was observed in the 1990s: peak egg abundance changed from April–June to end of July (Wieland et al. 2000, Kraus et al. 2002, Karasiova et al. 2008). Later spawning has consequences for several processes influencing survival of early life stages (MacKenzie et al. 1996, Støttrup et al. 2008). Identification of the mechanisms causing variations in spawning time is of great importance for understanding the recruitment dynamics of this stock. Ambient water temperature and fishery-induced changes in age composition can affect timing of spawning (Wright & Trippel 2009), but these factors cannot fully explain the delayed spawning observed in the Baltic Sea. Periods with low feeding condition are correlated with delayed spawning in Baltic cod (Baranova 1995, Karasiova et al. 2008), indicating that delayed spawning may also be diet related. Low energetic status, particularly lipid levels, can reduce the levels of hormones involved in gonadal development (Cerda et al. 1994, Matsuyama et al. 1994), and a recent study indicated that the composition of lipids, which differs among cod stocks, may influence maturation and spawning time in Baltic cod (Tomkiewicz et al. 2009).

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Lipids, and particularly fatty acids, are major dietary factors determining successful reproduction, and serve not only as the preferred source of metabolic energy for the parent fish, but are also crucial for the future requirements of the progeny (Sargent et al. 2002, Tocher 2003). Lipids are incorporated into oocyte yolk from dietary sources or from reserves that are stored prior to vitellogenesis, and are subsequently mobilized and transferred to the ovary (Wiegand 1996). Not only the quantity, but also the quality of dietary lipids is important, and long chain polyunsaturated fatty acids (PUFA) have been identified as a major dietary factor determining successful reproduction. The PUFAs docosahexaenoic acid (22:6n-3, DHA), eicosapentaenoic acid (20:5n-3, EPA) and arachidonic acid (20:4n-6, ARA) are especially important for egg and larval quality. Docosahexaenoic acid and EPA have a structural role in membrane phospholipids (PL), and EPA and ARA are precursors for eicosanoids including prostaglandins (Sargent et al. 1999b, Tocher 2003), which have a role in final oocyte maturation and ovulation in fish (Mustafa & Srivastava 1989, Goetz & Garczynski 1997, Sorbera et al. 2001, Lister & Van der Kraak 2008).

Due to their polyunsaturated nature, PUFAs are highly susceptible to attack by free radicals followed by a reaction with oxygen, which results in lipid peroxidation. The ease of lipid peroxidation is proportional to the number of double bonds present (Sargent et al. 2002). Peroxidation can have serious consequences for cell membrane structure and function, and highly efficient antioxidant protection is essential. α-tocopherol and carotenoids, particularly astaxanthin in cod (Miki et al. 1982, Grung et al. 1993), both have a protective antioxidative role in fish, quenching harmful excited oxygen and free radicals (Syvaoja et al. 1985, Miki 1991).

The majority of marine fishes do not possess the ability to synthesize either PUFAs or α-tocopherol and carotenoids themselves (Sargent et al. 2002). The extent to which fish can convert C_{18} fatty acids to $C_{20/22}$ varies with species, and is associated with their capacity for fatty acyl desaturation and elongation (Tocher 2003). Δ6 desaturase activities and PUFA biosynthesis are very low in cod hepatocytes and enterocytes, irrespective of diet (Tocher et al. 2006). As a consequence, the biosynthesis of PUFAs is very low in cod, and PUFA levels are therefore determined by the dietary intake. Hence, cod depend on PUFAs and tocopherols that are synthesized in phytoplankton or plants and transferred up through the food web, while astaxanthin is synthesized by crustaceans from carotenoid precursors provided by algae, especially β-carotene and zeaxanthin (Matsuno 2001).

Phytoplankton composition varied in the Baltic Sea between 1979 and 2005 due to increased temperature and decreased salinity and inorganic nitrogen concentrations (Wasmund & Uhlig 2003, Wasmund et al. 2011). Strong spring blooms of Diatomophyceae occurred in the 1980s and again from 2000, whereas those of Dinophyceae occurred in the 1990s. It is most likely that these changes have altered fatty acid composition (FAC) in the base of the food web, since FAC differs among phytoplankton groups (Ahlgren et al. 1992, Viso & Marty 1993, Dijkman & Kromkamp 2006). Elevated levels of n-3/n-6 fatty acids in phytoplankton and very high DHA/ARA ratios observed in copepods from the Baltic Sea compared to the Norwegian Sea in a mesocosm enrichment study from 2001 (Ahlgren et al. 2005) could indicate such changes taking place within the plankton community. The Baltic copepods are the main prey of Baltic sprat and herring (Möllmann et al. 2004), which are the most important prey of adult cod in the eastern Baltic Sea (Bagge et al. 1994, Neuenfeldt & Beyer 2003). The benthic crustacean *Saduria entomon* is also sporadically found in cod stomachs. Condition has decreased in Baltic herring since the late 1980s and in Baltic sprat since the 1990s due to increased food competition caused by changes in zooplankton composition concurrent with increased sprat abundance (Köster et al. 2005, Möllmann et al. 2005, Casini et al. 2006, 2010, 2011). The amount of mesenteric fat declined significantly in herring from 1985 to 1991 (Flinkman et al. 1998), likely reducing the nutritional quality of herring as prey for cod.

Differences in PUFAs were found in the gonad and liver of late maturing cod from the Baltic Sea compared to cod from the North Sea, suggesting that deficiencies in PUFAs might be the underlying cause for the delay in spawning time of cod (Tomkiewicz et al. 2009), because no delay in spawning is observed in North Sea cod. To investigate if PUFA requirements for cod reproduction are met, measurements of absolute levels are needed. Cod exhibit determinate fecundity and a multiple batch spawning strategy where vitellogenic oocytes successively undergo final maturation during the process of batch development (Tomkiewicz et al. 2003). Cod spawning can be extensive; Kjesbu (1989) found that captive North Sea cod spawned up to 17–19 batches over a period of 50 to 60 d. Stores of lipids and fatty acids are grad-

ually emptied during the reproductive process, potentially affecting egg quality over the spawning period.

The aim of the present study was to investigate variation in lipid content as well as proportions and absolute values of PUFAs of female Baltic cod gonads and livers during a complete reproductive cycle. In gonads we also investigated levels of α-tocopherol and astaxanthin to measure antioxidant protection. The liver is the primary storage site of lipid energy in cod (Kjesbu et al. 1991), and we examined whether the lipid composition of cod liver reflected its major prey, sprat and herring, and if it was possible to detect fatty acid 'finger prints' of *Saduria entomon.*

MATERIALS AND METHODS

Sample collection

Female cod were sampled on board trawl surveys and fisher boats in the central Baltic Sea in February, March, May, July and August 2009 (Fig. 1). The aim was to sample 5 to 10 females per length group per sampling event in the length classes 30–39, 40–49, 50–59 and >60 cm, to distribute samples over the most common size groups of reproducing females, including a minimum of 3 female cod of each prevailing maturity stage per sampling. For each female, total length (TL) was measured to the nearest lower integer (cm), whole body weight (BW) and eviscerated body weight (EBW, gonad, liver, stomach and viscera removed) was measured to the nearest g. Gonad weight (GW) and liver weight (LW) were measured to the nearest 0.1 g. Gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as: GSI = GW × 100/EBW and HSI = LW × 100/EBW. Gonadal maturity stages of cod (MIII = early ripening, MIV = late ripening, MV = initiation of spawning, MVI = main spawning, MVII = cessation of spawning, MVIII = spent and MIX = resting) were judged from macroscopic characters according to the method of Tomkiewicz et al. (2003) during sampling. A subsample of the ovarian tissue was preserved in formalin buffered with $NaH_2PO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 2H_2O$ for histological validation. The remaining ovarian tissue and liver were frozen and stored at –40°C for analysis of lipids, astaxanthin and tocopherol in the laboratory. Muscle dry weight was determined on board the research vessels or in the laboratory. Ten grams of fillet was dried in an oven in aluminium trays at 105°C and weighed (±0.001 g) after 18, 48 and 72 h. Sprat *Sprattus sprattus* and herring *Clupea harengus* were sampled in November 2008, February, March, May and August 2009, and the isopod *Saduria entomon* was sampled in March 2009 and November 2010. Total length of sprat and herring was measured to the nearest lower ½ cm and specimens were grouped according to length class. Sampled sprat, herring and isopods were stored at –40°C for later lipid analysis.

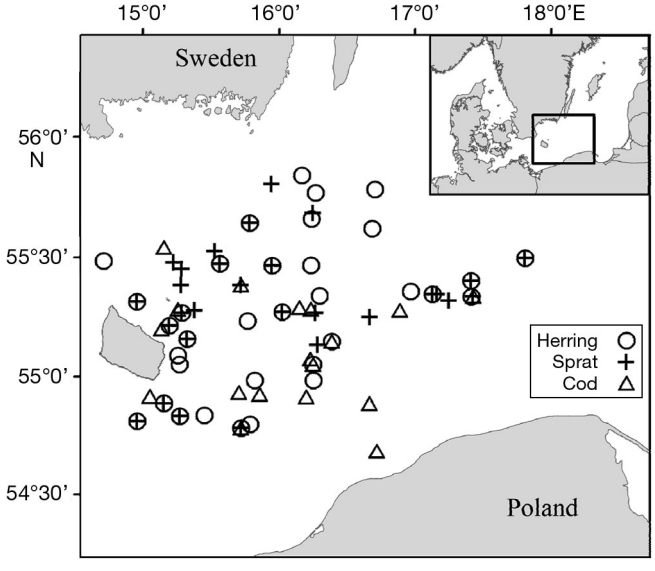

Fig. 1. Study area. Samples were collected in ICES subdivision 25 at or near the Bornholm Basin in the Baltic Sea. Symbols represent stations where the 3 species were sampled from November 2008 to August 2009

Histology

To verify the maturity stage of female cod, ovarian development was evaluated using histological sections. The preserved ovarian tissue was embedded in paraffin using standard procedures, sectioned at 7 µm and stained using hematoxylin and eosin. Sections were analyzed using light microscopy and maturity stages were distinguished on the basis of morphological characteristics of the larger and more advanced oocytes (Tomkiewicz et al. 2003). The early ripening stage (MIII) is a very diverse stage, and lipid composition may differ among sub-stages. MIII was therefore further divided into 3 sub-stages according to the characteristics of the most advanced oocytes: oocytes with cortical alveoli (CA) only = $MIII^0$; peripheral yolk granules among CA = $MIII^1$; and yolk granules fill most but not the entire cytoplasm = $MIII^2$ (Tomkiewicz et al. 2003).

Subsampling for lipid analysis

Only adult females ≥40 cm were chosen for lipid analysis. The histological classification regrouped the samples and the target minimum of 3 females of each maturity stage (or sub-stage of MIII) was not achieved in all sampling months; smaller sample sizes for lipid analysis were accepted in these cases. In cases of >3 females within maturity stages and sampling events, 3 samples were randomly chosen using Research Randomizer (www.randomizer.org/).

For each sampling month, sprat and herring from several stations were separated into 10 mm length classes and pooled (5 to 58 fish per sample) for lipid analysis. Five classes of sprat (80 to 130 mm) and 8 classes of herring (130 to 230 mm) were considered, representing the dominant size range found in cod stomachs (S. Neuenfeldt, pers. comm.). Specimens of *Saduria entomon* in various sizes were pooled according to sampling month and station for lipid analysis.

Lipid extraction and determination of lipid content

Sprat, herring and isopods were cut into small pieces, frozen using liquid nitrogen and blended. Lipids were extracted from 5 to 10 g of the blended samples. For cod, the samples of ovary and liver as well as 10 samples of muscle were analysed individually. The extraction was done with a homogeneous mixture of chloroform, methanol and water (2:2:1.8) following the method of Bligh & Dyer (1959). The method was modified to use a smaller volume of solvents, but the original ratio between chloroform, methanol and water was maintained. Lipid extracts were used for the subsequent lipid class fractionation and determination of FAC as well as for determination of lipid content. Lipid content was determined by gravimetry after evaporation of chloroform, and is expressed as percentage of wet weight of ovary and liver respectively.

Lipid class separation

Lipids from ovary and liver extracts were separated into phospholipids (PL) and neutral lipids (NL) by chromatography on a solid phase consisting of aminopropyl modified silica. Solvents with increasing polarity were used to separate lipid classes. A lipid extract corresponding to 10–100 mg lipid was used for the lipid class separation. Solvents from the lipid extraction were evaporated and the extract was resolubilized in 0.5 ml chloroform and transferred to a Sep-Pak column (Waters Corporation). Neutral lipids were eluted using 4 ml chloroform/2 ml propanol (2:1), and PL were eluated with 6 ml methanol. Eluates were evaporated until almost dry (NL) or to 1–2 ml (PL) under nitrogen. The only lipid class of NL present in cod that contains fatty acids is triacylglycerols (TAG), and this term is used throughout this paper.

Preparation of fatty acid methyl esters (FAME) and analysis of FAC

Eluates of ovary or liver extracts from the lipid class separation and extracts of total lipid from sprat herring and isopods were used for the preparation of FAME using AOCS Official Method Ce 2-66 (AOCS 1998b). FAME were analysed on a HP 5890A gas chromatograph (Hewlett-Packard) equipped with an Omegawax 320 (30 m × 3.2 mm × 0.25 µm) column (Supelco) using AOCS Official Method Ce 1b-89 (AOCS 1998a). The oven temperature programme was 15°C min^{-1} to 160°C, hold 2 min, 3°C min^{-1} to 200°C, hold 1 min, 3°C min^{-1} to 220°C, hold 17 min. A split ratio of 1:25 was used and C23:0 methyl ester was used as an internal standard. The proportion of fatty acids was quantified by calculating its peak area relative to the total peak area (% of total FA) and absolute content of fatty acids was calculated relative to the internal standard and equivalent sample size (mg g^{-1} wet wt). Absolute total content of fatty acids in ovarian and liver lipid (mg g^{-1} lipid) was estimated as the sum of absolute content of identified fatty acids (mg g^{-1} wet wt) in PL and TAG divided by the percentage of lipid content in ovary and liver respectively, assuming that the sum of unidentified fatty acids was more or less constant between samples.

Lipid class determination

Lipid classes were determined using thin-layer chromatography with flame ionization detection (TLC-FID) with Iatroscan MK5. Lipid extracts from gonad and liver were either concentrated or diluted to ensure a concentration ranging from 10 to 20 mg ml^{-1}. Triplicates of 1 µl samples of lipid extracts were spotted on silica gel coated Chromarods® (SIII) using an Autospotter (Model SES 3202). Chromarods® were developed in the following solvent system at 20°C: n-heptane/diethyl ether/formic acid

(70:10:0.02, vol:vol:vol) until solvent reach the 90% mark (around 20 min). The chromarods were then heated at 110°C for 5 min to dry the solvent off, and scanned in the Iatroscan. This single development separates the NL classes: TAG, free fatty acids, cholesterol and total PL. Data were analyzed using SES ChromStar software and peak areas were quantified using calibration curves obtained from scans of standards (trilinolein, lynoleic acid, cholesterol and L-alpha-phosphatidylcholin, Sigma-Aldrich).

Tocopherol and astaxanthin

Tocopherol was measured in cod ovary samples and in whole sprat and herring using AOCS Official Method Ce 8-89 (AOCS 1997). Samples of 2 g lipid extract were evaporated under oxygen-free nitrogen, re-dissolved in 1 ml heptanes and mixed for 30 s. Separation of tocopherols was performed by HPLC (Agilent 1100) equipped with a 3 µm Silica column (150 mm × 4.6 mm, Water Spherisorb). Concentration of tocopherol was calculated from the peak area relative to the internal standard (α, β, γ, δ standards, Calbiochem 613424) and equivalent sample size (µg g^{-1}). Astaxanthin was measured spectrophotometrically (UV mini 1240) at 485 nm in lipid extract from cod ovary samples and in whole sprat, herring and *Saduria entomon*, and concentration was calculated using the extinction coefficient $E_{1\%}$ = 2460 and equivalent sample size (µg g^{-1}).

Statistical analyses

Multivariate data analysis was performed using the Unscrambler® v9.1 (CAMO) software. Principal component analyses (PCA) were performed and variables included relative peak areas of 30 identified fatty acids, groups and ratios of fatty acids, HSI, GSI, lipid content, season and maturity stage. Variables were column mean centred and normalised to unit column standard deviation (SD) before calculation of the models. Models were validated by full cross-validation. Statistical differences in oil and fatty acid content were assessed with R version 2.12. The data were checked for normal distribution and tested with 1-way ANOVA. A comparison between Model 1 (ANOVA including only maturity stage) and Model 2 (ANOVA including both maturity stages and season) was tested with the Bonferroni correction. If no significant differences were found between the models, the simpler Model 1 was used to test differences between maturity stages, followed by a multiple comparison test. If significant differences were found, Model 2 was used. A significance level (p) of 0.05 was applied in all tests.

RESULTS

Sample characteristics

In total, 144 female cod were sampled. After histological validation, 51% of the samples were in developing condition (MIII and MIV), 20% in spawning stages (MV–MVII), 24% in spent or resting stages (MVIII and MIX) and 5% were immature (MII). Females in spawning stages were obtained during May, July and August, representing 50, 93 and 15%, respectively, of the females sampled within each sampling event, indicating the timing of peak spawning and duration of the spawning season. A subsample of 45 cod in maturity sub-stages $MIII^{0–2}$ and maturity stages MIV–MIX were chosen for lipid analysis (Table 1). Total length

Table 1. Subsample of female cod for analysis of lipid content and fatty acid composition (FAC). Number of specimens per maturity stage (MIII–MIX) after histological validation and total length (TL), body weight (BW) and water content (Water) in muscle samples. Values are means ± SD, n = 45. MIII: early ripening (3 sub-stages); MIV: late ripening; MV: initiation of spawning; MVI: main spawning; MVII: cessation of spawning; MVIII: spent; MIX: resting

Maturity	Feb	Mar	May	Jul	Aug	Total	TL (cm)	BW (kg)	Water (%)
$MIII^0$	3	3	0	0	0	6	55.2 ± 10.4	1.6 ± 0.7	82.0 ± 1.0
$MIII^1$	4	3	0	0	0	7	61.0 ± 18.8	2.6 ± 2.2	81.2 ± 0.7
$MIII^2$	0	3	0	0	0	3	48.3 ± 2.5	1.4 ± 0.8	81.0 ± 0.5
MIV	1	3	2	1	2	9	50.3 ± 6.8	1.4 ± 0.4	82.9 ± 3.1
MV	0	0	2	3	0	5	45.8 ± 4.5	1.2 ± 0.3	82.6 ± 2.0
MVI	0	0	0	3	0	3	50.7 ± 7.4	1.6 ± 1.0	84.8 ± 0.9
MVII	0	0	3	0	0	3	48.3 ± 5.1	1.2 ± 0.3	86.1 ± 3.7
MVIII	0	0	0	0	2	2	54.5 ± 7.8	1.6 ± 0.6	82.0 ± 0.6
MIX	4	0	0	0	3	7	57.0 ± 6.5	1.7 ± 0.5	82.2 ± 0.6

of the female cod for lipid analysis varied between 40 and 92 cm, with an average length of 53.1 cm and average weight of 1.6 kg. There were no significant differences in length and weight among sampled females among maturity stages or seasons (Table 1). The size range of sampled cod reflected the composition of spawners in the stock, i.e. the highest proportion is below 2 kg (ages 3 to 6 yr) in the sample area ICES sub-division 25 (ICES 2010). Water content in muscle was significantly higher in fish at the end of spawning (86% in MVII) than at early maturation (81% in $MIII^1$) (Table 1). Due to windy weather in May, it was not possible to use the fine balance on board and the lower accuracy of the balance used (±1 g) resulted in a high variation in muscle water content of maturation stage MIV, MV and MVII. The lipid content of fillets was on average 0.66%. Fatty acid composition in TAG of cod liver in the different months, in relative and absolute content, is shown in Appendix 1. In total, 623 sprat and 641 herring were included in this study. They were pooled into 45 sprat and 62 herring samples, and *Saduria entomon* were pooled into 4 samples.

Multivariate analysis

Principal components analysis of stored TAG in liver showed that FAC in liver varied with maturation; Principal Component (PC) 1 explained 22% and PC2 explained an additional 15% of the variation in samples (Fig. 2). Samples of spawning female cod (MV and MVI) were grouped together, except for 1 outlier of MVI (Fig. 2a). Likewise, samples of cod at the end of spawning (MVII) and resting (MIX) were relatively similar; however, some samples from the 2 groups overlapped. FAC of maturing (MIII and MIV) and resting (MVIII) fish was highly variable; however, MIII samples were mainly located on the left side of the plot. The corresponding loadings plot (Fig. 2b) shows that liver TAG of cod in MIII was characterized by high ARA, 18:1n-7 and 20:1n-7, and that DHA, total n-3 PUFA and EPA/ARA were highest in liver of MVII and MIX cod, while monounsaturated fatty acids (MUFA) were low.

Principal components analysis of ovary PL shows that FAC and other sample characteristics varied with maturation, indicating cyclic dynamics with gonadal maturation (Fig. 3a). Principal Component 1 explained 27% and PC2 explained an additional 16% of the variation in samples. Fatty acid content of fish in early maturation ($MIII^0$ and $MIII^1$) was similar to the composition of resting fish (MIX) in the 2nd and 3rd quadrant, while fish in late maturation (MIV) were situated close to the spawning cod (MV and MVI) in the 1st and 4th quadrant, except for 1 outlier. Fish at the end of the early maturation stage ($MIII^2$) and end of spawning (MVII) and spent (MVIII) stages were grouped in transition zones (dotted lines). The corresponding loadings plot (Fig. 3b) shows that ovary PL of cod in early maturation, particularly $MIII^0$, were characterized by high levels of 16:1n-7, 24:1n-9, 14:0, 15:0, 16:0 and total saturated fatty acids (SFA), and cod in the resting stage (MIX) were characterized by high levels of ARA, 18:3n-6, total n-6 PUFA and 16:2n-4. Ovary PL in late maturation and spawning were characterized by high GSI, lipid content and high levels of DHA, n-3 PUFA, PUFA, 18:1n-9, total MUFA and tocopherol.

Lipid dynamics and gonadal maturation

Variation in FAC in PL and TAG of liver and ovary in the different maturation stages is shown in Tables 2 & 3. The ANOVA model including both maturity stage (including sub-stages of MIII) and season was not statistically different from the ANOVA model with maturity stage alone for any variable. The only effect of season on FAC was therefore indirect (through maturity stage), and so ANOVA Model 1 was applied to Tables 2 & 3. More fatty acids differed significantly between maturation stages in PL of ovary and liver than in TAG of both organs, where only a few fatty acids differed significantly. The sum of SFAs, dominated by 16:0, did not vary with maturation in any lipid fraction, but was in general higher in PL than in TAG in both ovary and liver. The sum of MUFAs, dominated by 18:1n-9, only varied with maturation in ovary PL and was much higher in TAG than PL of both ovary and liver. The sum of PUFAs varied significantly with maturation in PL and TAG of ovaries, but not in livers, and was higher in PL than TAG of both ovary and liver.

The proportion of DHA increased with gonadal maturation and decreased again after spawning in ovary PL and TAG and liver PL, but did not vary with maturation stage in liver TAG. The proportion of ARA decreased significantly from early to late maturation and/or spawning in all lipid fractions, increased significantly again after spawning in ovary PL, and tended also to increase in ovary TAG. Eicosapentaenoic acid, on the other hand, did not vary sig-

nificantly with maturity stage in any lipid fraction. The decrease in ARA resulted in an increase in EPA/ARA ratios in late maturation in ovary PL and TAG and liver PL.

Gonadosomatic index varied significantly with maturity stage from an average of 2.8 in Stage MIII^0 to a maximum of 45 in Stage MVI, followed by a decrease to 1.8 in Stage MIX (Fig. 4a). Lipid content

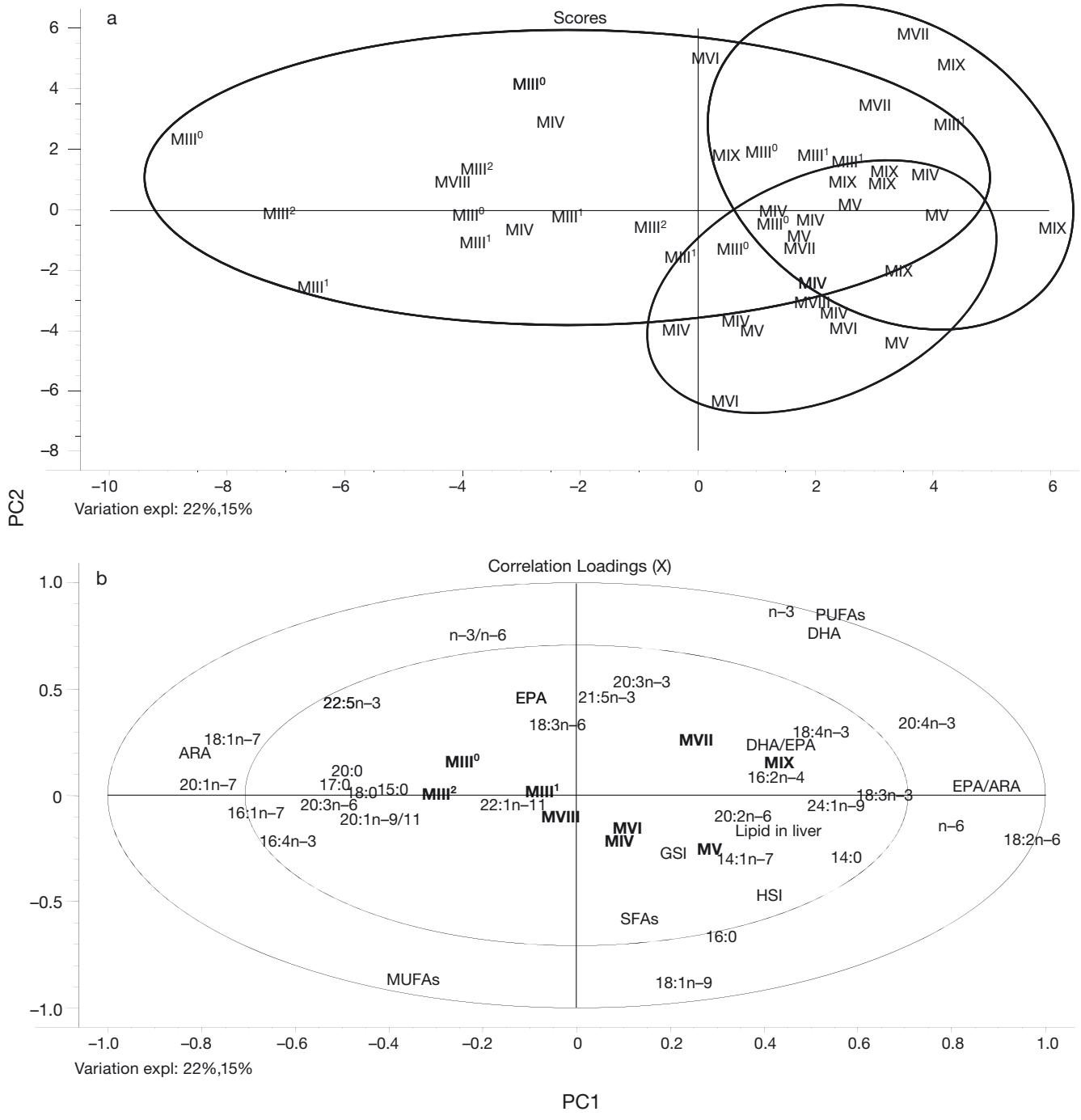

Fig. 2. Principal component analysis (PCA): (a) scores plot and (b) loadings plot of liver triacylglycerol (TAG) including fatty acid composition (FAC), lipid content in liver, hepatosomatic index (HSI), gonadosomatic index (GSI), maturity sub-stages MIII$^{0–2}$ and maturity stages MIV–MIX, n = 45. Ellipses in (a) illustrate clustering of samples and in (b) the inner ellipse indicates 50% explained variance and the outer ellipse 100% explained variance. SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ARA: arachidonic acid

in ovaries peaked at 4% in MIV. Average HSI and lipid content in the liver (Fig. 4b) did not vary significantly when all maturation stages were considered, but reached the lowest levels in MVI. The amount of lipid in ovaries relative to lipid in liver ([GW × lipid content] / [LW × lipid content]) increased significantly with maturation, from 2.5 on average in $MIII^0$ to 21 in MV, and decreased to 1 in MIX. The average

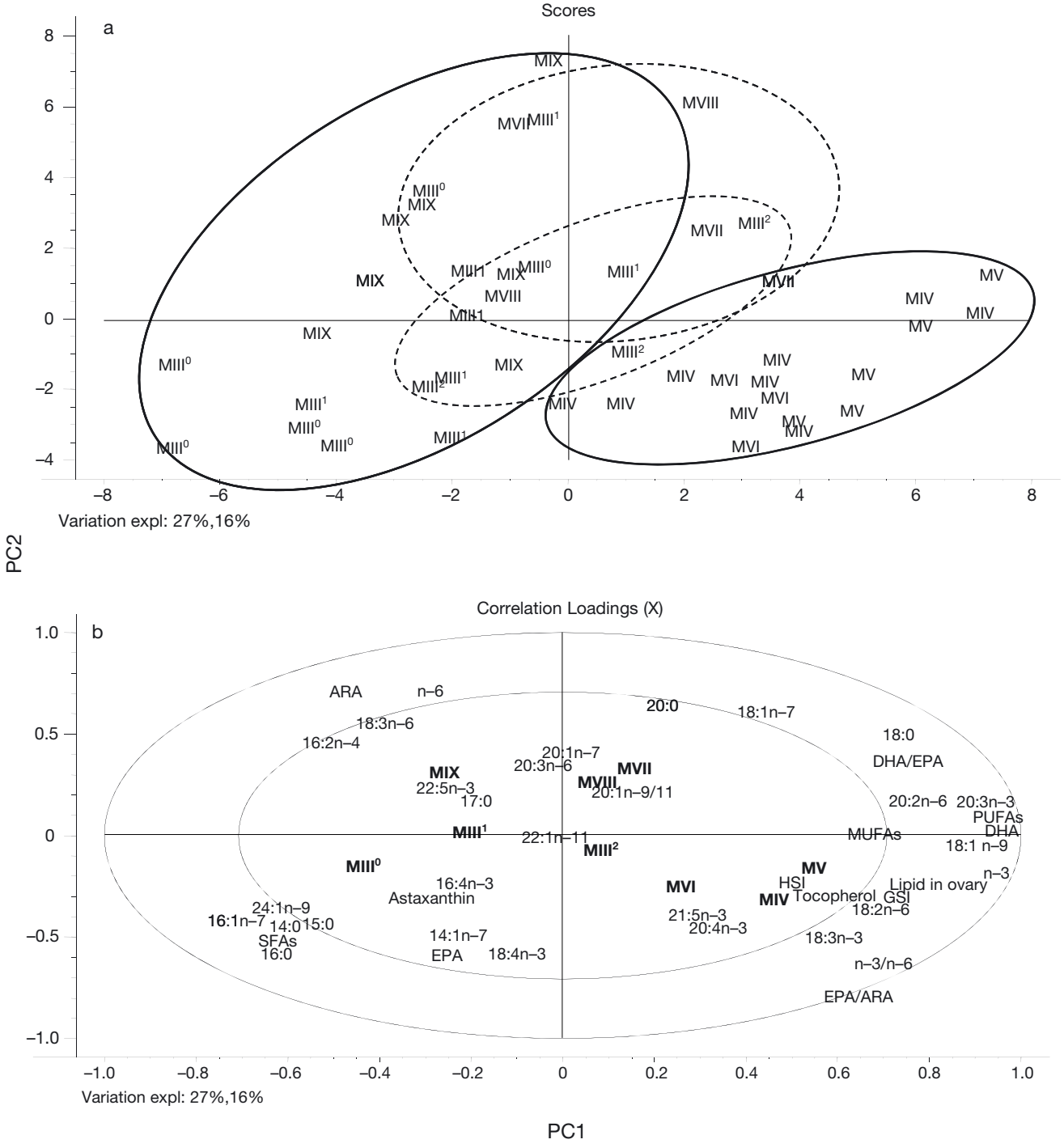

Fig. 3. Principal component analysis (PCA): (a) scores plot and (b) loadings plot of ovarian phospholipid (PL) including fatty acid composition (FAC), lipid content in ovary, hepatosomatic index (HSI), gonadosomatic index (GSI), astaxanthin, α-tocopherol, maturity sub-stages $MIII^{0–2}$ and maturity stages MIV–MIX, n = 45. Ellipses in (a) illustrate clustering of samples; continuous lines illustrate main clusters of samples and dashed lines illustrate subset of samples in transition zones. In (b) the inner ellipse indicates 50% explained variance and the outer ellipse 100% explained variance. SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ARA: arachidonic acid

proportion of PL in gonads increased to 88% in MVI, while the proportion of TAG decreased to 3% (Fig. 4c). Average proportions of cholesterol varied between 7 and 40%, and free fatty acids (FFA) between 1 and 8% (not shown). In livers, the average proportion of TAG was 89% and PL 7%, with no variation with maturation (Fig. 4d). Cholesterol and FFA varied between 0 and 4% (not shown).

Table 2. Fatty acid composition (% of total fatty acids) and fatty acid ratios of phospholipids (PL) and triacylglycerols (TAG) in ovaries from Baltic cod in maturity sub-stages MIII$^{0-2}$ and maturity stages MIV–MIX. Values are means ± SD. Fatty acids with <1.5% in both PL and TAG of ovary are excluded. Values with different superscripts within rows are statistically different, $p < 0.05$

Fatty acid	MIII^{0}	MIII^{1}	MIII^{2}	MIV	MV	MVI	MVII	MVIII	MIX
Phospholipids									
14:0	1.5 ± 0.4	1.2 ± 0.4	1.0 ± 0.4	1.0 ± 0.3	0.9 ± 0.4	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.5	1.6 ± 0.4
16:0	22.4 ± 2.5	21.2 ± 2.6	20.0 ± 3.2	20.1 ± 2.4	18.9 ± 2.2	21.1 ± 0.6	19.9 ± 2.0	20.1 ± 3.2	21.1 ± 1.9
18:0	1.1 ± 0.3^{b}	1.2 ± 0.3^{ab}	1.6 ± 0.4^{bc}	1.9 ± 0.6^{bc}	2.3 ± 0.7^{c}	1.6 ± 0.2^{bc}	2.5 ± 0.3^{c}	2.4 ± 0.7^{ac}	1.9 ± 0.4^{bc}
SFAs	26.0 ± 2.9	24.4 ± 2.9	23.4 ± 3.4	23.6 ± 2.2	22.7 ± 2.0	24.5 ± 0.3	23.9 ± 2.1	23.9 ± 3.2	25.3 ± 2.0
16:1n-7	3.7 ± 1.2^{a}	3.1 ± 0.5^{ab}	2.5 ± 0.7^{ac}	2.0 ± 0.6^{bc}	1.7 ± 0.5^{c}	2.0 ± 0.3^{bc}	1.3 ± 0.0^{c}	1.5 ± 0.2^{bc}	2.2 ± 1.0^{bc}
18:1n-9	8.0 ± 0.9^{a}	7.9 ± 0.7^{a}	9.7 ± 1.7^{ab}	11.2 ± 1.8^{bc}	12.5 ± 0.7^{b}	11.7 ± 1.8^{bc}	12.6 ± 2.0^{b}	11.2 ± 0.4^{ab}	9.2 ± 1.4^{ac}
18:1n-7	2.1 ± 0.5	2.4 ± 0.8	2.7 ± 0.7	2.4 ± 0.3	2.6 ± 0.2	2.4 ± 0.7	2.8 ± 0.4	3.5 ± 2.1	2.3 ± 0.5
24:1n-9	1.2 ± 0.3^{b}	1.1 ± 0.4^{ab}	0.8 ± 0.3^{bc}	0.7 ± 0.2^{ac}	0.6 ± 0.1^{c}	0.6 ± 0.2^{ac}	0.4 ± 0.1^{c}	0.7 ± 0.4^{bc}	0.8 ± 0.2^{bc}
MUFAs	15.8 ± 1.5^{ab}	15.3 ± 1.1^{a}	16.4 ± 1.4^{ab}	17.2 ± 1.2^{ab}	17.9 ± 0.5^{b}	17.2 ± 1.3^{ab}	17.8 ± 1.5^{ab}	17.6 ± 1.5^{ab}	15.2 ± 1.3^{a}
16:2n-4	1.4 ± 0.3^{bd}	1.5 ± 0.3^{ab}	1.0 ± 0.2^{acd}	0.9 ± 0.2^{cd}	0.7 ± 0.1^{c}	0.9 ± 0.1^{acd}	1.5 ± 0.6^{bd}	1.4 ± 0.7^{bc}	1.9 ± 0.3^{b}
18:2n-6	0.8 ± 0.2^{b}	0.8 ± 0.2^{ab}	0.9 ± 0.1^{bc}	1.3 ± 0.2^{ac}	1.5 ± 0.4^{c}	1.5 ± 0.3^{c}	1.1 ± 0.2^{bc}	1.2 ± 0.5^{bc}	1.1 ± 0.4^{bc}
18:3n-3	0.4 ± 0.0^{ab}	0.4 ± 0.0^{a}	0.5 ± 0.1^{ab}	0.5 ± 0.1^{ab}	0.5 ± 0.1^{ab}	0.6 ± 0.1^{b}	0.4 ± 0.1^{ab}	0.4 ± 0.1^{ab}	0.4 ± 0.2^{ab}
18:4n-3	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.2	0.3 ± 0.1
20:4n-6	3.2 ± 0.7^{bc}	2.8 ± 1.1^{ab}	2.9 ± 0.1^{ab}	1.8 ± 0.2^{a}	1.6 ± 0.2^{a}	1.8 ± 0.1^{ac}	2.9 ± 0.8^{ab}	4.0 ± 1.0^{b}	3.7 ± 1.0^{b}
20:5n-3	13.7 ± 2.3	12.7 ± 2.8	14.1 ± 2.1	13.3 ± 1.0	12.1 ± 0.7	13.0 ± 0.8	11.3 ± 2.1	13.1 ± 2.0	12.6 ± 1.0
22:5n-3	0.9 ± 0.2	1.0 ± 0.4	0.9 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	0.7 ± 0.1
22:6n-3	26.0 ± 4.7^{a}	29.9 ± 4.3^{ab}	30.9 ± 4.0^{ab}	33.5 ± 2.4^{b}	35.6 ± 2.0^{b}	33.2 ± 0.6^{ab}	31.7 ± 1.8^{ab}	28.5 ± 3.9^{ab}	28.1 ± 2.0^{a}
PUFAs	48.2 ± 3.6^{a}	50.8 ± 2.0^{ab}	53.0 ± 1.9^{ab}	53.7 ± 1.9^{b}	54.7 ± 1.5^{b}	53.6 ± 1.8^{b}	51.2 ± 2.6^{ab}	51.4 ± 1.5^{ab}	50.6 ± 2.4^{ab}
n-6	4.7 ± 0.7^{bc}	4.3 ± 1.0^{ab}	4.4 ± 0.4^{bc}	3.7 ± 0.2^{b}	3.8 ± 0.3^{b}	3.9 ± 0.2^{ab}	4.8 ± 0.9^{bc}	6.1 ± 0.4^{ac}	5.9 ± 1.3^{c}
n-3	42.1 ± 3.5^{b}	45.0 ± 1.9^{abc}	47.6 ± 1.7^{cd}	49.1 ± 1.9^{d}	50.1 ± 1.5^{d}	48.9 ± 1.7^{ad}	44.9 ± 4.0^{bd}	43.9 ± 1.8^{bd}	42.8 ± 1.8^{bc}
DHA/EPA	2.0 ± 0.6	2.5 ± 0.9	2.3 ± 0.6	2.5 ± 0.3	3.0 ± 0.3	2.6 ± 0.1	2.9 ± 0.4	2.2 ± 0.6	2.3 ± 0.3
DHA/ARA	8.4 ± 2.4^{b}	12.1 ± 4.9^{ab}	10.7 ± 1.0^{b}	18.9 ± 2.9^{c}	22.6 ± 2.4^{c}	18.5 ± 1.1^{ac}	11.5 ± 3.4^{ab}	7.2 ± 0.9^{b}	7.9 ± 1.8^{b}
EPA/ARA	4.4 ± 1.3^{b}	5.0 ± 1.8^{ab}	4.9 ± 0.9^{bc}	7.4 ± 0.6^{c}	7.7 ± 1.0^{c}	7.3 ± 0.8^{ac}	4.2 ± 1.7^{ab}	3.4 ± 1.4^{b}	3.6 ± 1.0^{b}
Triacylglycerols									
14:0	1.9 ± 0.3	1.8 ± 0.3	1.6 ± 0.4	1.6 ± 0.3	1.6 ± 0.3	1.7 ± 0.6	1.3 ± 0.1	1.6 ± 0.9	2.1 ± 0.6
16:0	16.0 ± 3.4	18.5 ± 2.7	15.2 ± 3.6	17.1 ± 2.4	18.6 ± 1.6	15.2 ± 2.0	20.6 ± 0.8	17.7 ± 6.0	19.0 ± 4.2
18:0	1.1 ± 0.3^{ac}	1.0 ± 0.3^{a}	1.5 ± 0.3^{ab}	1.5 ± 0.2^{ab}	1.4 ± 0.2^{ab}	1.9 ± 0.4^{bc}	1.6 ± 0.2^{ab}	2.1 ± 0.6^{b}	1.6 ± 0.4^{ab}
SFAs	20.1 ± 3.0	22.1 ± 2.4	19.2 ± 2.9	20.9 ± 2.4	22.4 ± 1.8	19.5 ± 1.3	24.2 ± 0.8	22.0 ± 4.5	23.3 ± 3.3
16:1n-7	6.2 ± 1.0^{a}	6.1 ± 0.7^{a}	5.4 ± 2.0^{ab}	3.9 ± 1.1^{bc}	2.9 ± 0.3^{c}	4.0 ± 1.2^{ac}	2.2 ± 0.1^{c}	3.0 ± 0.7^{bc}	3.4 ± 1.1^{bc}
18:1n-9	16.5 ± 4.2	15.2 ± 4.4	18.0 ± 3.4	17.3 ± 2.8	15.6 ± 1.2	19.6 ± 6.0	15.2 ± 1.4	15.9 ± 5.0	14.8 ± 2.4
18:1n-7	3.1 ± 0.8	2.6 ± 0.8	3.5 ± 1.3	2.4 ± 0.7	2.1 ± 0.3	3.2 ± 0.8	2.0 ± 0.3	3.0 ± 0.1	2.1 ± 0.4
24:1n-9	2.3 ± 0.4^{ad}	2.6 ± 0.7^{a}	1.6 ± 0.6^{ab}	1.2 ± 0.3^{bc}	0.7 ± 0.4^{b}	0.9 ± 0.4^{bc}	0.6 ± 0.3^{b}	0.9 ± 0.6^{bcd}	2.1 ± 0.8^{ac}
MUFAs	29.4 ± 5.7	27.5 ± 5.6	29.6 ± 6.9	25.9 ± 3.8	21.9 ± 1.1	28.8 ± 7.6	20.7 ± 1.2	24.0 ± 6.8	23.6 ± 3.5
16:2n-4	0.8 ± 0.1	0.9 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	1.0 ± 0.2	0.8 ± 0.1	0.9 ± 0.3
18:2n-6	1.2 ± 0.4	1.2 ± 0.3	1.4 ± 0.2	1.8 ± 0.6	2.0 ± 0.5	2.6 ± 0.9	1.5 ± 0.2	2.1 ± 1.5	2.1 ± 1.1
18:3n-3	0.8 ± 0.2	0.7 ± 0.2	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.2	1.5 ± 0.4	0.8 ± 0.0	1.2 ± 0.9	1.2 ± 0.7
18:4n-3	0.6 ± 0.2	0.5 ± 0.2	0.7 ± 0.1	0.7 ± 0.3	0.7 ± 0.2	1.1 ± 0.5	0.5 ± 0.1	0.9 ± 0.8	1.1 ± 0.7
20:4n-6	2.6 ± 0.7^{a}	2.4 ± 1.0^{ab}	2.1 ± 0.5^{ab}	1.5 ± 0.2^{b}	1.3 ± 0.2^{b}	1.2 ± 0.3^{ab}	2.0 ± 0.4^{ab}	2.4 ± 1.2^{ab}	2.4 ± 0.7^{ab}
20:5n-3	11.8 ± 2.5	12.3 ± 1.3	11.7 ± 2.0	11.6 ± 0.9	11.2 ± 0.5	10.1 ± 1.9	11.5 ± 0.7	12.1 ± 0.9	13.0 ± 1.2
22:5n-3	1.1 ± 0.3	1.2 ± 0.4	1.1 ± 0.3	0.8 ± 0.3	0.8 ± 0.1	1.1 ± 0.3	0.8 ± 0.1	1.0 ± 0.2	0.9 ± 0.1
22:6n-3	19.9 ± 2.2^{a}	20.1 ± 2.5^{a}	22.1 ± 4.5^{abc}	26.8 ± 2.8^{cd}	30.6 ± 1.1^{d}	26.0 ± 6.8^{ad}	29.5 ± 2.1^{bd}	25.3 ± 5.1^{ad}	22.6 ± 2.1^{ac}
PUFAs	40.8 ± 4.0^{b}	41.1 ± 3.3^{ab}	43.1 ± 6.0^{bc}	47.0 ± 2.5^{ac}	50.2 ± 1.4^{c}	46.2 ± 7.8^{bc}	49.5 ± 1.7^{c}	47.9 ± 3.5^{bc}	46.1 ± 2.4^{bc}
n-6	4.3 ± 0.5^{ab}	4.1 ± 0.8^{a}	4.1 ± 0.3^{ab}	3.9 ± 0.5^{a}	3.9 ± 0.5^{a}	4.4 ± 0.7^{ab}	4.2 ± 0.5^{ab}	5.2 ± 0.5^{ab}	5.2 ± 0.8^{b}
n-3	35.7 ± 3.7^{a}	36.1 ± 3.1^{a}	38.3 ± 5.5^{ab}	42.4 ± 2.6^{b}	45.6 ± 1.2^{b}	41.2 ± 8.3^{ab}	44.3 ± 2.4^{b}	41.9 ± 4.1^{ab}	40.0 ± 2.0^{ab}
DHA/EPA	1.7 ± 0.4^{a}	1.6 ± 0.1^{a}	1.9 ± 0.1^{ab}	2.3 ± 0.3^{bc}	2.7 ± 0.2^{c}	2.6 ± 0.4^{c}	2.6 ± 0.1^{c}	2.1 ± 0.3^{ac}	1.7 ± 0.2^{a}
DHA/ARA	8.2 ± 2.4^{b}	9.4 ± 3.4^{ab}	10.5 ± 0.6^{ab}	18.4 ± 2.8^{cd}	23.6 ± 3.4^{c}	21.3 ± 1.0^{ce}	15.2 ± 4.4^{ade}	11.1 ± 3.2^{bd}	9.8 ± 2.5^{ab}
EPA/ARA	4.7 ± 0.7^{a}	5.7 ± 1.8^{a}	5.6 ± 0.4^{abc}	7.9 ± 0.6^{bd}	8.6 ± 1.1^{d}	8.5 ± 1.2^{cd}	5.9 ± 1.6^{ad}	5.5 ± 2.3^{ad}	5.7 ± 1.4^{ac}

Average DHA content in ovary lipid increased to 171 mg g^{-1} lipid in MV and decreased again in MIX (Fig. 4e). In contrast, ARA decreased to around 8 mg g^{-1} lipid in MIV and during spawning, and then increased again, while EPA was more or less constant. In liver lipid, absolute content of DHA was relatively stable until an increase in MVII, while ARA decreased as early as MIV and was only ~5 mg g^{-1}

Table 3. Fatty acid composition (% of total fatty acids) and fatty acid ratios of phospholipids (PL) and triacylglycerols (TAG) in livers from Baltic cod in maturity sub-stages $MIII^{0-2}$ and maturity stages MIV–MIX. Values are means ± SD. Fatty acids with <1.5% in both PL and TAG of liver are excluded. Values with different superscripts within rows are statistically different, $p < 0.05$

Fatty acid	$MIII^0$	$MIII^1$	$MIII^2$	MIV	MV	MVI	MVII	MVIII	MIX
Phospholipids									
14:0	1.6 ± 0.3^{bc}	2.1 ± 0.5^{ab}	1.5 ± 0.0^{bc}	1.8 ± 0.4^{bc}	1.3 ± 0.2^{c}	1.1 ± 0.1^{c}	1.3 ± 0.3^{ac}	1.4 ± 0.5^{bc}	2.4 ± 0.6^{b}
16:0	20.0 ± 0.6^{ab}	19.4 ± 1.1^{ab}	19.7 ± 0.4^{ab}	20.3 ± 1.0^{ab}	21.0 ± 0.8^{b}	21.5 ± 1.6^{b}	21.3 ± 1.1^{b}	18.1 ± 0.4^{a}	19.8 ± 1.5^{ab}
18:0	5.6 ± 0.9^{ab}	6.1 ± 1.6^{a}	4.6 ± 0.5^{ab}	4.6 ± 1.1^{ab}	3.5 ± 0.6^{b}	3.9 ± 1.5^{ab}	3.6 ± 0.6^{ab}	4.4 ± 2.0^{ab}	5.1 ± 1.6^{ab}
SFAs	28.3 ± 1.1	29.0 ± 3.2	26.7 ± 0.4	27.4 ± 1.7	26.5 ± 0.8	27.2 ± 0.7	26.9 ± 0.8	24.6 ± 3.0	28.5 ± 2.4
16:1n-7	1.3 ± 0.2^{ab}	1.5 ± 0.7^{ab}	1.2 ± 0.1^{ab}	1.2 ± 0.4^{ab}	0.9 ± 0.2^{a}	0.9 ± 0.1^{ab}	1.4 ± 0.4^{ab}	1.2 ± 0.2^{ab}	1.9 ± 1.0^{b}
18:1n-9	10.0 ± 0.9	10.0 ± 1.1	10.7 ± 1.0	11.1 ± 1.5	11.9 ± 1.0	10.7 ± 1.9	10.9 ± 1.8	9.9 ± 1.3	9.7 ± 2.9
18:1n-7	2.5 ± 0.6^{ab}	2.5 ± 0.8^{ab}	2.8 ± 0.4^{ab}	2.5 ± 0.5^{ab}	2.4 ± 0.1^{ab}	2.5 ± 0.3^{ab}	2.6 ± 0.1^{ab}	3.6 ± 1.2^{a}	2.0 ± 0.3^{b}
24:1n-9	0.3 ± 0.2^{bc}	0.3 ± 0.1^{ab}	0.2 ± 0.1^{ac}	0.1 ± 0.1^{c}	0.2 ± 0.0^{ac}	0.2 ± 0.0^{ac}	0.3 ± 0.1^{bc}	0.3 ± 0.1^{bc}	0.4 ± 0.1^{b}
MUFAs	14.9 ± 1.4	15.3 ± 1.9	15.3 ± 0.8	15.6 ± 1.6	15.8 ± 1.2	14.6 ± 1.6	15.5 ± 1.7	15.5 ± 0.4	14.6 ± 4.3
16:2n-4	0.7 ± 0.2^{a}	0.5 ± 0.1^{ab}	0.5 ± 0.1^{ab}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.1^{bc}	0.6 ± 0.1^{ac}	0.5 ± 0.1^{ab}	0.6 ± 0.1^{ac}
18:2n-6	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.2	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	1.2 ± 0.1	1.4 ± 0.6	1.7 ± 0.6
18:3n-3	0.5 ± 0.2^{ab}	0.3 ± 0.3^{a}	0.4 ± 0.3^{ab}	0.7 ± 0.2^{ab}	0.6 ± 0.1^{ab}	0.6 ± 0.1^{ab}	0.5 ± 0.1^{ab}	0.6 ± 0.1^{ab}	0.9 ± 0.6^{b}
18:4n-3	0.4 ± 0.1^{ab}	0.5 ± 0.1^{ab}	0.3 ± 0.1^{b}	0.3 ± 0.2^{b}	0.3 ± 0.1^{b}	0.2 ± 0.1^{b}	0.3 ± 0.1^{b}	0.3 ± 0.1^{ab}	0.7 ± 0.3^{a}
20:4n-6	2.6 ± 0.6^{b}	2.4 ± 0.7^{ab}	1.9 ± 0.2^{bc}	1.3 ± 0.2^{c}	1.5 ± 0.2^{ac}	1.8 ± 0.4^{bc}	1.7 ± 0.3^{bc}	2.5 ± 1.4^{bc}	1.9 ± 0.4^{bc}
20:5n-3	14.3 ± 0.7^{ab}	13.7 ± 1.8^{ab}	13.2 ± 2.1^{ab}	12.1 ± 0.7^{ab}	11.4 ± 0.5^{a}	11.7 ± 1.0^{ab}	13.3 ± 3.3^{ab}	15.9 ± 3.2^{ab}	14.9 ± 2.6^{b}
22:5n-3	0.7 ± 0.2	0.8 ± 0.5	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.2	0.6 ± 0.3	0.5 ± 0.2
22:6n-3	30.4 ± 1.5^{ab}	29.7 ± 2.9^{ab}	34.1 ± 1.2^{bc}	35.0 ± 2.1^{c}	36.8 ± 1.1^{c}	36.9 ± 1.7^{c}	34.2 ± 2.0^{bc}	32.3 ± 1.4^{ac}	28.3 ± 3.3^{a}
PUFAs	52.2 ± 1.2	50.7 ± 2.1	53.9 ± 1.5	53.3 ± 1.5	54.3 ± 0.5	54.8 ± 2.4	53.8 ± 2.6	55.9 ± 2.9	51.4 ± 4.4
n-6	4.4 ± 0.5^{ac}	4.4 ± 0.7^{a}	3.6 ± 0.4^{ab}	3.2 ± 0.3^{bc}	3.4 ± 0.4^{b}	3.7 ± 0.4^{ab}	3.5 ± 0.4^{ab}	4.5 ± 0.8^{ac}	4.4 ± 0.5^{a}
n-3	47.2 ± 1.2^{ab}	45.8 ± 2.2^{a}	49.8 ± 1.4^{ab}	49.7 ± 1.5^{b}	50.5 ± 0.7^{b}	50.6 ± 1.9^{ab}	49.6 ± 2.2^{ab}	50.8 ± 2.2^{ab}	46.4 ± 4.1^{ab}
DHA/EPA	2.1 ± 0.2^{a}	2.2 ± 0.4^{a}	2.6 ± 0.5^{ab}	2.9 ± 0.3^{b}	3.2 ± 0.2^{ab}	3.2 ± 0.4^{ab}	2.7 ± 0.8^{ab}	2.1 ± 0.5^{ab}	1.9 ± 0.4^{ab}
DHA/ARA	12.4 ± 3.3^{b}	13.3 ± 3.4^{ab}	18.5 ± 2.5^{bc}	26.2 ± 2.7^{c}	25.5 ± 3.5^{c}	20.6 ± 3.9^{ac}	20.4 ± 3.7^{bc}	15.4 ± 9.3^{ab}	15.2 ± 3.4^{ab}
EPA/ARA	5.8 ± 1.4^{a}	6.2 ± 1.8^{a}	7.1 ± 0.6^{ab}	9.1 ± 1.1^{b}	7.9 ± 0.8^{ab}	6.6 ± 1.8^{ab}	7.8 ± 1.5^{ab}	7.1 ± 2.8^{ab}	7.9 ± 1.7^{ab}
Triacylglycerols									
14:0	2.3 ± 0.3	2.4 ± 0.5	2.0 ± 0.5	2.4 ± 0.6	2.8 ± 0.4	2.3 ± 0.7	2.4 ± 0.1	2.2 ± 0.2	2.9 ± 0.4
16:0	13.2 ± 0.9	13.4 ± 0.9	13.0 ± 0.4	13.2 ± 0.9	14.0 ± 1.2	13.9 ± 1.9	12.7 ± 1.1	13.0 ± 0.4	13.9 ± 0.8
18:0	3.1 ± 0.4	3.2 ± 0.6	3.2 ± 0.7	2.8 ± 0.5	2.5 ± 0.4	2.9 ± 0.6	2.2 ± 0.5	2.9 ± 0.6	2.3 ± 0.3
SFAs	19.6 ± 0.9	19.7 ± 1.5	19.3 ± 0.8	19.2 ± 1.0	20.0 ± 1.9	19.8 ± 2.0	18.0 ± 1.4	18.8 ± 1.2	19.8 ± 1.1
16:1n-7	5.3 ± 0.9	5.3 ± 1.5	5.5 ± 0.3	4.7 ± 0.6	4.5 ± 0.2	4.9 ± 0.4	4.3 ± 0.2	5.2 ± 1.3	4.5 ± 0.3
18:1n-9	20.6 ± 3.5	21.6 ± 2.7	21.9 ± 2.3	23.7 ± 4.0	24.1 ± 2.9	23.3 ± 6.8	20.8 ± 5.0	23.1 ± 5.1	20.8 ± 3.0
18:1n-7	4.3 ± 1.0	3.9 ± 0.9	4.4 ± 0.8	3.5 ± 0.7	3.3 ± 0.2	4.1 ± 0.6	3.3 ± 0.4	4.5 ± 1.0	3.3 ± 0.3
24:1n-9	0.6 ± 0.2	0.6 ± 0.3	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.2
MUFAs	33.2 ± 2.9	34.0 ± 4.8	34.6 ± 1.2	35.3 ± 2.8	34.2 ± 2.3	34.9 ± 6.5	30.3 ± 4.5	36.2 ± 1.1	31.1 ± 2.7
16:2n-4	0.5 ± 0.1^{b}	0.6 ± 0.0^{ab}	0.5 ± 0.1^{b}	0.5 ± 0.1^{b}	0.6 ± 0.1^{ab}	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}	0.6 ± 0.0^{ab}	0.6 ± 0.1^{ab}
18:2n-6	2.7 ± 0.8	2.8 ± 0.6	2.5 ± 0.7	3.3 ± 0.7	3.8 ± 0.7	3.5 ± 0.8	3.4 ± 0.3	3.3 ± 1.0	3.7 ± 0.8
18:3n-3	2.2 ± 0.5	2.2 ± 0.6	2.1 ± 0.3	2.0 ± 0.8	2.5 ± 0.1	2.3 ± 0.2	2.3 ± 0.1	2.2 ± 0.5	2.4 ± 0.4
18:4n-3	1.7 ± 0.5	1.7 ± 0.5	1.6 ± 0.3	1.9 ± 0.4	1.8 ± 0.3	1.8 ± 0.4	1.6 ± 0.0	1.4 ± 0.3	1.8 ± 0.3
20:4n-6	1.2 ± 0.4^{a}	0.9 ± 0.2^{ab}	1.2 ± 0.2^{ab}	0.8 ± 0.2^{b}	0.7 ± 0.1^{b}	0.8 ± 0.1^{ab}	0.7 ± 0.0^{ab}	0.8 ± 0.3^{ab}	0.7 ± 0.2^{b}
20:5n-3	9.6 ± 1.8	8.6 ± 2.0	9.9 ± 0.8	8.7 ± 1.2	7.9 ± 0.6	7.1 ± 1.4	8.7 ± 0.5	7.8 ± 0.3	8.7 ± 1.2
22:5n-3	1.5 ± 0.5	1.5 ± 0.7	1.6 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	1.5 ± 0.6	1.7 ± 0.5	1.8 ± 0.8	1.3 ± 0.3
22:6n-3	20.1 ± 2.9	20.5 ± 3.0	18.4 ± 0.7	19.7 ± 2.2	20.1 ± 2.3	20.4 ± 6.1	25.4 ± 5.4	19.2 ± 2.6	22.7 ± 3.5
PUFAs	42.4 ± 3.2	41.6 ± 5.4	40.7 ± 1.4	40.9 ± 3.1	41.4 ± 3.2	40.8 ± 8.0	47.2 ± 5.7	40.0 ± 1.6	44.8 ± 2.9
n-6	4.8 ± 0.5	4.6 ± 0.4	4.6 ± 0.6	4.9 ± 0.5	5.4 ± 0.8	5.2 ± 0.6	4.9 ± 0.3	5.0 ± 0.7	5.3 ± 0.8
n-3	37.1 ± 3.0	36.4 ± 5.1	35.6 ± 0.8	35.5 ± 2.9	35.5 ± 3.2	35.0 ± 8.2	41.6 ± 5.4	34.4 ± 2.3	38.9 ± 3.0
DHA/EPA	2.2 ± 0.6	2.5 ± 0.5	1.9 ± 0.2	2.3 ± 0.3	2.5 ± 0.3	2.9 ± 0.6	3.0 ± 0.8	2.5 ± 0.4	2.7 ± 0.7
DHA/ARA	18.6 ± 7.5^{a}	24.2 ± 9.1^{ab}	16.2 ± 3.5^{ab}	26.5 ± 6.6^{ab}	28.0 ± 5.2^{ab}	25.7 ± 3.1^{ab}	35.0 ± 9.0^{b}	24.0 ± 6.1^{ab}	31.5 ± 6.9^{b}
EPA/ARA	8.4 ± 1.8	10.2 ± 4.0	8.7 ± 1.9	11.5 ± 2.1	11.0 ± 1.6	9.2 ± 1.9	11.8 ± 0.7	10.1 ± 4.3	12.2 ± 3.2

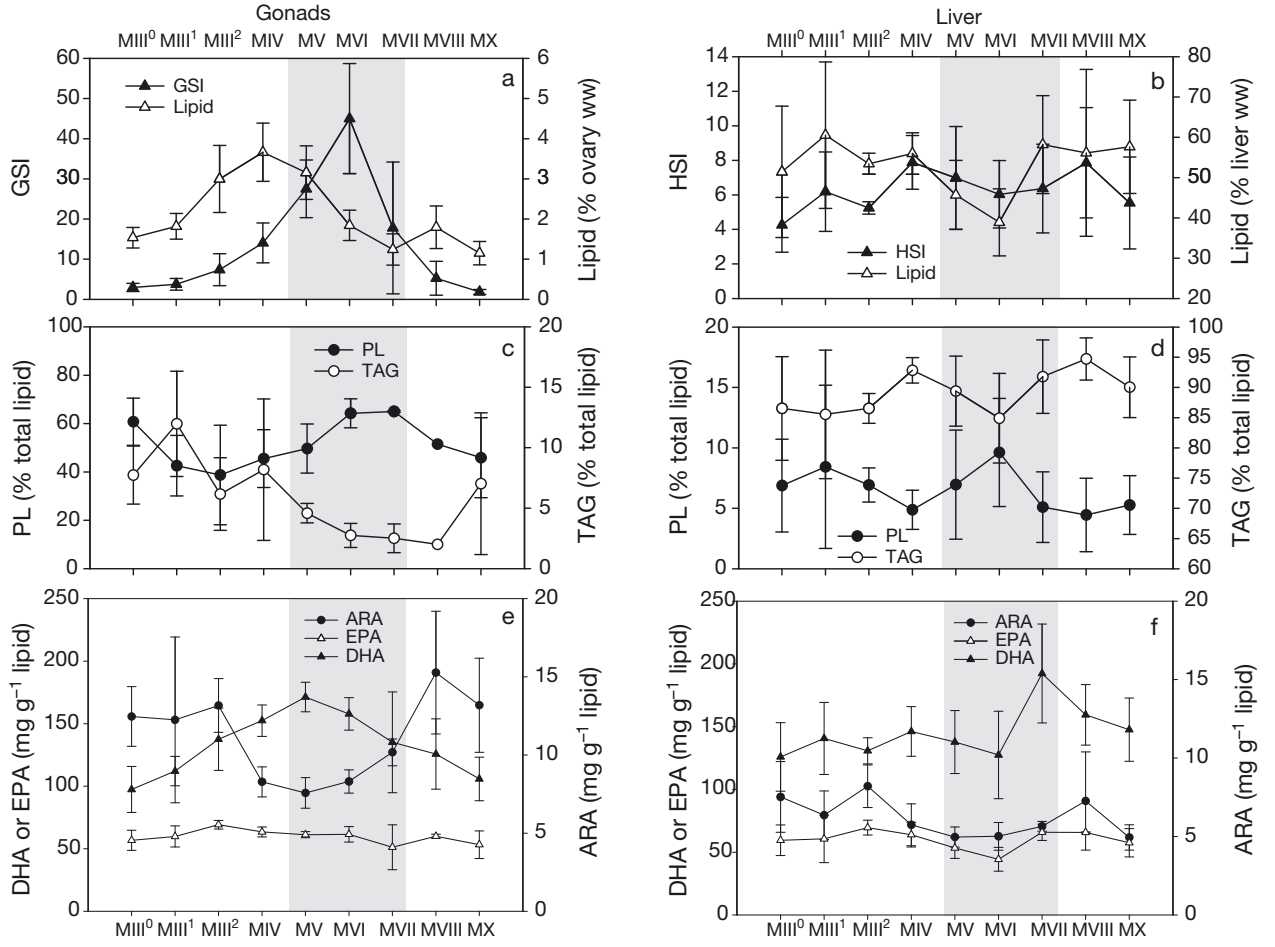

Fig. 4. Variation in gonadosomatic index (GSI), hepatosomatic index (HSI) and lipid parameters in female cod (n = 45) across maturity sub-stages $MIII^{0-2}$ and maturity stages MIV–MIX. (a) GSI and ovarian lipid content. (b) HSI and liver lipid content. (c,d) Proportion of phospholipids (PL) and triacylglycerols (TAG) in (c) ovary (4 samples were excluded from this analysis due to technical problems) and (d) liver. (e,f) Estimated content of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) in total lipids (sum of PL and TAG) in (e) ovarian lipid and (f) liver lipid. Values are means ± SD. Grey boxes represent the maturity stages with spawning fish. ww: wet weight

lipid during spawning, and EPA was relatively constant (Fig. 4f). Arachidonic acid in ovary lipid was positively correlated (slope = 1.0) with ARA in the liver ($r^2 = 0.25$, $p < 0.01$) (Fig. 5), while no correlation was found for DHA ($r^2 = 0.01$, $p < 0.01$) and EPA ($r^2 = 0.00$, $p < 0.01$) (not shown).

Predator–prey relationships

Fatty acid content of cod liver TAG and FA content of total lipid in herring, sprat and *Saduria entomon* were compared to identify predator–prey relationships (Fig. 6). The proportion of 18:0, 16:1n-7, 18:1n-7, and ARA were higher in cod liver than in sprat and herring, while 16:0 and 18:3n-3 were

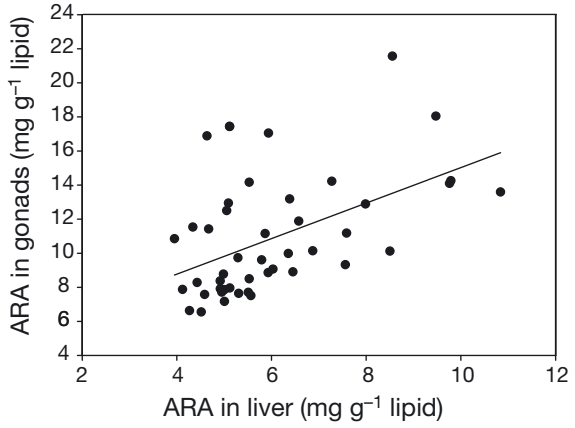

Fig. 5. Estimated contents of arachidonic acid (ARA) of total lipids (sum of phospholipids and triacylglycerols) in ovary as a function of estimated contents in liver, n = 45

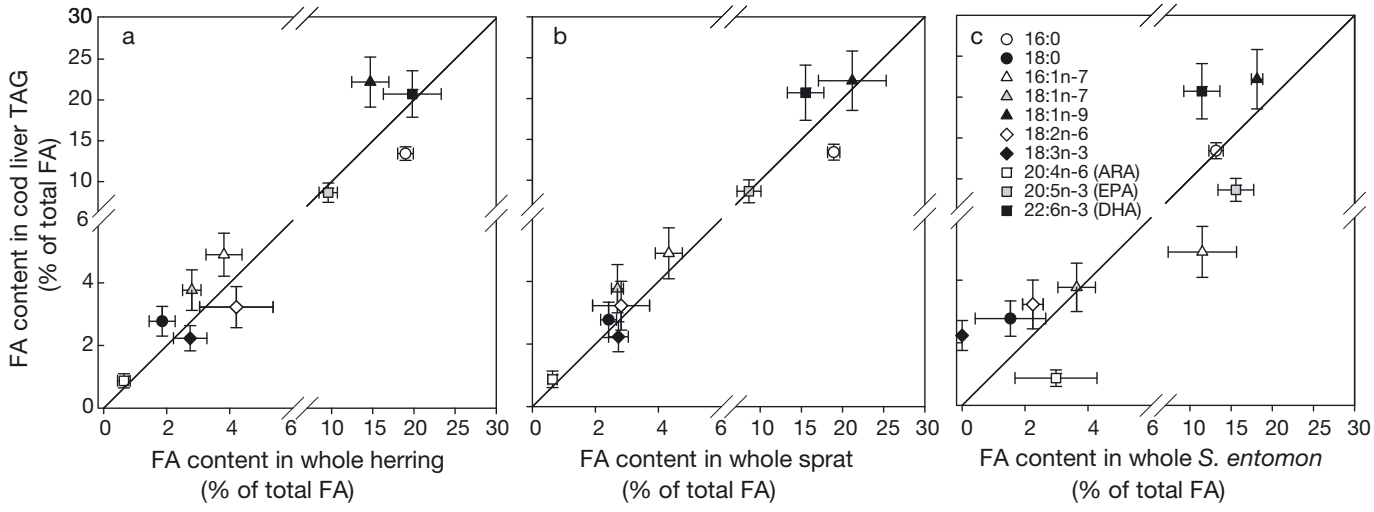

Fig. 6. Relationship between mean (± SD) fatty acid (FA) content of cod liver triacylglycerols (TAG) and mean (± SD) FA content of total lipid in (a) whole herring *Clupea harengus*, (b) sprat *Sprattus sprattus* and (c) *Saduria entomon*. Values are the mean of all seasons and the line illustrates line of equality. Cod: n = 45; herring: n = 62; sprat: n = 45; *S. entomon*: n = 4

lower. The average proportion of 18:1n-9 in cod was similar to the content in sprat, while level of DHA was similar to that of herring. The level of 18:2n-6 in cod was higher than in sprat, but lower than in herring. The proportions of 16:0 and 18:1n-7 were identical in cod liver and *S. entomon*, while 16:1n-7, EPA and ARA were found in higher proportions in *S. entomon*.

Antioxidants in cod and its prey

The only tocopherol homologue detected in ovaries was α-tocopherol. The concentration of α-tocopherol in gonads at different maturity stages is shown in Fig. 7a. The average concentration of α-tocopherol varied between 18.5 and 76.5 µg g^{-1}, with highest levels in late maturing cod. The average concentration of astaxanthin varied between 1.2 and 10.3 µg g^{-1} (Fig. 7b), but only $MIII^0$ and MIX differed significantly. The outlier in $MIII^0$ had an astaxanthin level of 27.6 µg g^{-1}; this ovary had a clear orange colour. The level of tocopherol was closely correlated with the level of lipid in ovaries ($r^2 = 0.73$, $p < 0.01$) while astaxanthin showed no relation to lipid level ($r^2 = 0.004$, $p = 0.67$) (Fig. 7c). The average ± SD astaxanthin concentration was 1.2 ± 0.6 µg g^{-1} in sprat (n = 45), 1.3 ± 0.7 µg g^{-1} in herring (n = 64) and 10.1 ± 5.4 µg g^{-1} in *Saduria entomon* (n = 3) and average α-tocopherol concentration was 1.7 ± 3.4 µg g^{-1} in sprat and 0.9 ± 2.1 µg g^{-1} in herring. No significant correlations between lipid level and astaxanthin and α-tocopherol concentration were found in sprat and herring.

DISCUSSION

Investment of lipid energy in reproduction

Cod invested a substantial amount of lipids in reproduction in the present study. Lipid transferred from the liver to the ovaries increased the amount of lipid in ovarian tissue relative to liver 10-fold during gonadal development, due to both the increase in size and lipid content of the ovaries. However, the food intake seemed to sustain high levels of liver energy, because cod had ample lipid energy, and liver lipid did not decrease until late in the reproductive cycle. This is later than is usually reported for wild cod: in Baltic cod, a 45% decrease in liver lipid was detected from early (MIII) to late (MIV) maturation (Shatunovskiy 1971) and a pronounced decrease in lipid was found before spawning from winter to spring in Atlantic cod from Balsfjorden in Norway (Eliassen & Vahl 1982) and Nova Scotia in Canada (Jangaard et al. 1967). The high lipid content in livers at the end of spawning (MVII) in the present study was not expected. The histological evaluation reclassified females of this maturity stage sampled in July and August, when this stage is normally most abundant, and only females from May remained for lipid analysis. Late spawning females are not common in the eastern Baltic in May; therefore, we cannot rule out the possibility that these fish originated from the Western Baltic cod stock that have peak spawning in February to April (Bagge et al. 1994, Bleil & Oeberst 1997).

Average GSI and HSI were similar to earlier reports for this stock (e.g. Tomkiewicz et al. 2003).

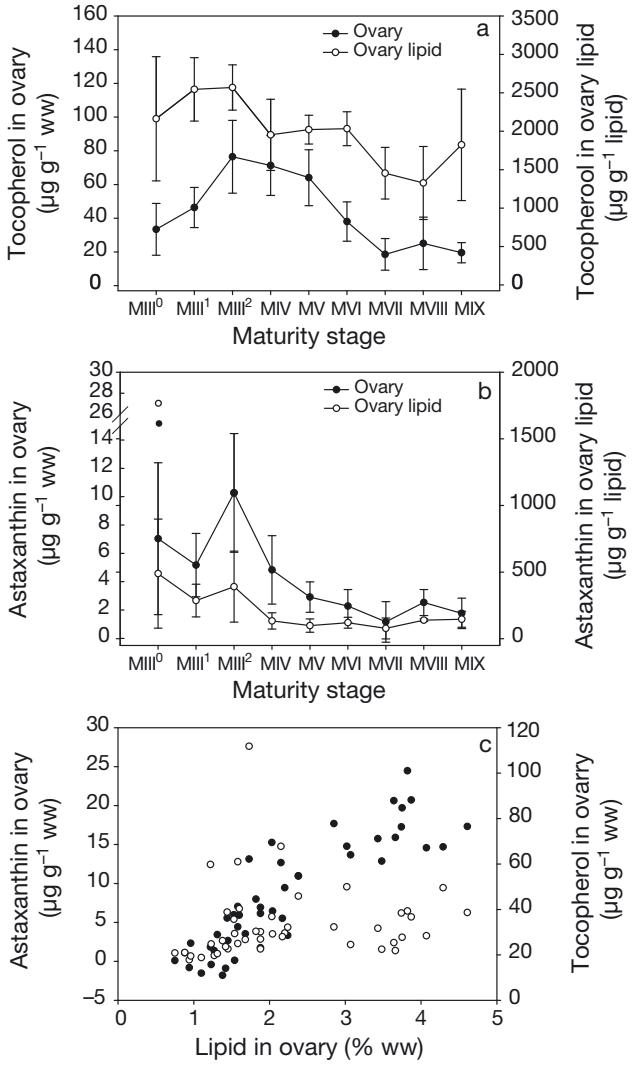

Fig. 7. Content of (a) α-tocopherol and (b) astaxanthin in ovaries of cod in maturity sub-stages $MIII^{0-2}$ and maturity stages MIV–MIX and (c) α-tocopherol (●) and astaxanthin (○) as a function of lipid content in ovaries. Values are means ± SD, n = 45

Hepatosomatic index tended to decrease from the spent (MVIII) to resting stage (MIX) in the present study, which could indicate that those fish had 'skipped' spawning, because this has previously been correlated with low HSI compared to a high HSI in resting fish which had spawned (Tomkiewicz et al. 2003). Also note that there are some discrepancies between the trend in HSI and lipid content, most likely due to variation in water content in the liver, which was previously reported for Norwegian cod (Eliassen & Vahl 1982). The amount of lipid energy stored in the liver is crucial for recruitment, as a highly significant linear relationship between total egg production and total lipid energy was observed in the Barents Sea cod stock (Marshall et al. 1999). The increased proportion of PL during spawning in both ovary and liver was most likely caused by a selective retention of PL and a selective catabolism of neutral lipids, especially TAG for reproduction, particularly egg production. Similarly, PL increased with GSI in Baltic herring and farmed trout (Kaitaranta & Ackman 1981). The variation in proportion of PL during a complete reproductive cycle is not yet described in cod; an increase in absolute content of PL from MIII to MIV and in relative content from MIV to MV was reported for Baltic cod (Shatunovskiy 1971). Phospholipids accounted for 64% of total lipids in ovaries during main spawning in the present study, which is comparable with earlier studies of cod, where phospholipids accounted for over 70% in ripe ovaries (Tocher & Sargent 1984) and in eggs and larvae (Fraser et al. 1988).

Selective incorporation of PUFA in ovaries

This study demonstrated a selective incorporation of PUFAs in ovaries during ovarian maturation. The PCA plots of ovary PL (Fig. 3) showed a gradual shift from (1) high levels of SFAs in early maturation, to (2) high lipid content, high levels of n-3 PUFA and low levels of n-6 PUFA in spawning fish, and (3) a shift back towards an immature state after spawning. The PCA plots also confirmed that PUFAs contributed significantly to the differences observed between spawning and non-spawning cod. The proportion of PUFAs was highest in PL compared to TAG, which is consistent with an earlier study of ovaries of cod and other marine fish species (Tocher & Sargent 1984) and is due to the structural role of PL in cell membranes (Sargent et al. 2002). The increased absolute content of DHA in ovary lipid in late maturation and spawning indicated a selective retention of DHA during ovarian development. The ratio of absolute content of both DHA and EPA in ovary and liver lipid was close to 1:1 during maturation, but increased in MV and MVI, which indicated a selective transfer of these PUFAs from liver to gonad during spawning. Furthermore, the increased DHA/EPA ratio in both ovary and liver tissue during late maturation and spawning indicated a selective catabolism of EPA relative to DHA in fatty acid oxidative processes, producing energy for oogenesis as suggested by Sargent et al. (2002). The increase in absolute content of EPA, and particularly DHA, in liver lipid in females at the end of spawning (MVII) could be attributed to inclusion of Western stock cod in the samples, as explained above.

The role of ARA in maturation processes

Low ARA content and high ratios of EPA to ARA coincided with cod ovarian development. ARA decreased in ovarian lipid in late maturation and spawning, in contrast to an increase in DHA. However, a high absolute content of ARA in ovary lipid compared to liver lipid, together with the strong correlation between absolute ARA levels in lipid of the gonad and liver, provided strong evidence for selective incorporation of this fatty acid into ovaries. The mechanisms underlying lipid deposition in maturing oocytes are not clearly understood, but the link between low levels of ARA and delayed maturation may be evident during oocyte maturation, with the lack of sufficient essential fatty acids for transfer to individual oocytes slowing down the process. However, this needs further verification in experimental studies *in vivo* and *in vitro*.

The decrease in ARA resulted in an increased EPA/ARA ratio in late maturation, which could have a negative effect on the activity of eicosanoids. Eicosanoids produced from ARA are more biologically active than those produced from EPA, and thus eicosanoid action is determined by the ratio of ARA to EPA in cellular membranes (Sargent et al. 2002). The mean proportions of ARA of 1.5 and 1.8% in TAG and PL, respectively, of ovaries in late maturation (MIV) in the present study were lower than the levels of 2.1 and 3.0% observed in ovaries of North Sea cod in 2004, and the EPA/ARA ratio of 7 to 8 in Baltic cod was higher than the ratio of 5 to 6 in North Sea cod, which does not have delayed spawning (J. Tomkiewicz unpubl. data). However, mean ARA and EPA/ARA in cod from the North Sea around Scotland in 1984 were similar to Baltic cod (Tocher & Sargent 1984). Arachidonic acid of 2.7% in total lipids of cod from Nova Scotia (Jangaard et al. 1967), which primarily spawn in winter, was also higher than in the Baltic; however, EPA/ARA was 8, similar to Baltic cod. However, the 2 latter studies were based on very few specimens, and the study of Jangaard et al. (1967) reported high individual variability. Hence, due to these contradictions and the low number of reference data, the effect of ARA and EPA/ARA on ovarian maturation and spawning time remains unresolved.

A DHA/EPA/ARA ratio around 15/8/1 is recommended for larval nutrition (Sargent et al. 1999a). The composition of ovaries in the present study did not deviate substantially from this ratio. However, the low ARA level in cod ovaries in late maturation and spawning could have negative effects on egg quality, as similar ARA levels in eggs were correlated with poor quality in cod (Pickova et al. 1997, Salze et al. 2005). In rearing experiments, hatching rate was significantly lower for eggs of Baltic cod than those of Skagerrak cod (Pickova & Larsson 1992, Larsson 1994, Pickova et al. 1997), indicating poor egg and larval quality of Baltic cod. Poor larval survival was observed in the mid-1990s and was related to the lack of recovery of the Baltic cod stock despite improved hydrographic conditions in this period (Köster et al. 2005). Hence, the low ARA level observed in cod may have affected egg and larval viability in the Baltic Sea. However, this needs further verification in experimental studies.

Other factors influencing spawning time

Low temperatures delay spawning in cod. Experimental results for Atlantic cod report a delay in spawning time by 8 to 10 d with a decrease in temperature of 1°C (Kjesbu 1994), and cod kept at low temperatures (4°C below ambient) showed a delay in spawning of 1 mo compared to cod kept at ambient temperatures (Kjesbu et al. 2010). Seasonal peak egg abundance in Baltic cod was also negatively associated with ambient water temperature in January to April in the period from1986 to 1996, but only at low levels of cod spawning biomass (Wieland et al. 2000). In the succeeding period of 1996 to 2005, spawning stock biomass was low and temperature relatively stable (ICES 2007). However, the date of peak egg abundance still varied considerably (R. Voss pers. comm.). Time series of temperature in the central Baltic Sea showed an increasing trend, and the annual temperature minimum in intermediate winter water increased by 1.5°C since 1988 (Meier et al. 2006). Therefore, temperature did not fully explain the shift in spawning time. In contrast, high temperatures were suggested to delay spawning time in halibut *Hippoglossus hippoglossus,* another coldwater species (Brown et al. 2006). However, according to the study by Wieland et al. (2000), this seems unlikely to be the case in Baltic cod.

Changes in age composition may influence the timing of spawning, because many fish species spawn progressively earlier within a season and may produce more egg batches over a longer period as they get older (Wright & Trippel 2009). This phenomenon was observed in cod (Marteinsdottir & Björnsson 1999, Tomkiewicz & Köster 1999), but the timing of peak spawning of Baltic cod females did not differ substantially relative to size (Tomkiewicz & Köster

1999, Tomkiewicz & Kraus 2005). Hence, it was concluded that changes in age composition were unlikely to be a major cause of delayed spawning.

Predator–prey relationships

The approach of tracing trophic pathways by comparing TAG of storage lipid and total lipid of whole prey followed the recommendations of Iverson (2009). Fatty acids of whole captive cod were found to reflect their diet after 3 wk or less, as no samples were taken earlier (Kirsch et al. 1998). In cod, dietary lipids are primarily stored in the liver (Lambert & Dutil 1997), and like most marine predators, cod consume their prey whole. Thus, liver samples most likely reflect dietary content earlier than muscle samples. The present results indicated a selective catabolism of 16:0 and selective incorporation of DHA, ARA, 18:1n-9 and 18:1n-7 in cod liver TAG. This is consistent with SFAs being predominantly mobilized for metabolic purposes, whereas 18:1 (Weber et al. 2003) and PUFAs are mainly retained (Sargent et al. 2002, Dalsgaard & St John 2004). Furthermore, low SFAs can be due to low assimilation, because the digestibility of fatty acids decreases with saturation (Koven et al. 1994, Olsen et al. 2004). The average proportion of 18:1n-9 and DHA in cod liver compared to proportions in sprat and herring indicated a mixed diet consisting of these 2 clupeids. The ratio of 18:1n-9 to DHA was almost twice as high in sprat than in herring and could function as a biomarker indicating the ratio of sprat and herring in cod diet.

The difference in FAC between sprat and herring was most likely due to differences in prey selectivity. In spring, both sprat and herring prey mainly on copepods, but in addition, sprat feed on cladocerans in summer and autumn (Möllmann et al. 2004), and larger herring favour mysids, amphipods and polychaetes, especially in autumn and winter (Casini et al. 2004). Herring were observed to switch from feeding mainly on adult stages of *Pseudocalanus* sp. to feeding mainly on young stages of *Temora longicornis* with increasing competition from sprat (Möllmann & Köster 2002). DHA was very abundant in *T. longicornis* compared to *Pseudocalanus* sp., which were very rich in 18:1n-9 (Peters 2006, Peters et al. 2006), which could explain the difference in the 18:1n-9/DHA ratio between sprat and herring.

The level of 16:1n-7 could indicate a trace of the crustacean *Saduria entomon* in cod diet. Dietary 16:1n-7 is a good lipid biomarker because it does not seem to be altered by marine animals. The content of this FA does not enter the internal biosynthetic pathway in copepods as it may only be elongated to longer-chain (n-7) isomers (Sargent & Henderson 1986), which are generally not detected in large amounts in calanoid copepods (Sargent & Falk-Petersen 1988). This is probably the same in fish, as indicated by a study where cod larvae fed monocultures of either diatom or dinoflagellates reflected the 16:1n-7/16:0 ratio of the phytoplankton after 13 d (St John & Lund 1996). We would therefore expect this fatty acid to be similar in cod liver and prey. However, the average proportion of 16:1n-7 was slightly higher in cod than its main prey, sprat and herring, which indicated a small proportion of *S. entomon* in the diet of Baltic cod. *S. entomon* was an important prey in the 1980s, when it contributed up to 30% of food consumption in the Central Baltic Sea (Zalachowski 1985, Uzars 1994), but it declined by at least a factor of 2 between 1985 and 1990 (Uzars 1994). The hypoxic zone in the Baltic Sea has increased in area about 4-fold since 1960 (Zillen et al. 2008) and abundance of *S. entomon* was low in hypoxic areas (Janas et al. 2004). A low proportion of *S. entomon* in cod diet is most likely to affect the ARA level in cod.

Antioxidant dynamics

The present study indicated an accumulation of α-tocopherol (vitamin E) during early ovarian development. Changes in tocopherol levels in cod ovaries with maturation have not yet been published, but the concentration in this study was on average 46× higher than in cod muscle from the northern part of the Baltic Sea (Syvaoja et al. 1985). An accumulation of α-tocopherol in ovaries is consistent with transport from muscle to ovary during vitellogenesis, as indicated in a study of salmon (Lie et al. 1994). The average concentration of α-tocopherol in cod ovaries was 12 and 89× greater than the Baltic herring and sprat respectively, and hence an accumulation in ovary compared to dietary concentration was evident. However, in a feeding trial with Atlantic cod, vitamin E concentration in muscle was not correlated with dietary vitamin E level, but was instead negatively correlated with dietary lipid content (Hemre et al. 2004). Higher dietary vitamin E concentrations significantly decreased muscle concentrations of thiobarbituric acid reactive substances, which are an index of lipid peroxidation and oxidative stress. We suggest that tocopherol was actively transported to the ovaries during vitellogenesis, and the decrease in concentration from late maturation ($MIII^2$) to end of

spawning (MVII) was caused by the antioxidant protection activity due to the increased levels of DHA.

Astaxanthin levels in cod ovaries were higher than in whole sprat and herring, indicating selective retention in the ovaries. This result is consistent with an accumulation of astaxanthin in ovaries compared to other tissues found in a recent study on Baltic cod (Nie et al. 2011). Astaxanthin levels were much higher in stomachs of sprat and herring compared to muscle, indicating that a direct transfer of crustacean astaxanthin from the clupeid stomach to piscivores is important (Nie et al. 2011). Astaxanthin levels in clupeids are therefore influenced by diet, and transfer of astaxanthin to higher trophic levels is consequently low in periods of starvation. However, the measured astaxanthin concentration in whole sprat and herring in the present study was approximately 10× higher than calculated from the weighted concentration of gonad, liver, muscle and stomach by Nie et al. (2011). This could indicate that a substantial proportion of astaxanthin is found in other body parts of clupeid such as skin, brain, kidneys and plasma, as reported for cod and salmon (Bell et al. 2000, Ytrestoyl & Bjerkeng 2007). *Saduria entomon* had on average 8× more astaxanthin than sprat and herring in the present study, as do many other Baltic crustaceans (Czeczuga 1976). Crustaceans can thus be considered an important dietary component, and consequently, a decreased intake of crustaceans would affect astaxanthin levels. The concentration of astaxanthin was on average 17× lower than α-tocopherol, but the defense mechanisms of astaxanthin were observed to be 100× greater (Miki 1991). Low astaxanthin levels during late maturation and spawning may cause inadequate antioxidant protection during maturation, which is critical due to high levels of PUFAs.

CONCLUSIONS

This study demonstrated an increase in relative lipid content in ovaries of Baltic cod during ovarian development and a notable decrease during maturation. Fatty acid content of the ovary and liver varied with maturity stage, and selective retention of DHA and ARA in ovaries during ovarian maturation was evident. Despite mobilization of ARA from the liver, the level of ARA in ovaries decreased in late maturation and during spawning. Low ARA content and a high ratio of EPA to ARA coincided with cod ovarian development in the central Baltic Sea. The antioxidants α-tocopherol and astaxanthin were accumulated in cod ovaries compared to prey, and decreased in late maturation and spawning due to antioxidant protection activity. The novel information on linkages between reproduction and lipid dynamics in Baltic cod provided in this manuscript is important because it enhances the understanding of lipid requirements in cod. However, experimental investigations of the detailed role of ARA in gonadal maturation, ovulation and for egg and larval quality in cod are needed to fully understand the underlying physiological processes involved.

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LITERATURE CITED

Ahlgren G, Gustafsson IB, Boberg M (1992) Fatty acid content and chemical composition of freshwater microalgae. J Phycol 28:37–50

Ahlgren G, Nieuwerburgh LV, Wänstrand I, Pedersén M, Boberg M, Snoeijs P (2005) Imbalance of fatty acids in the base of the Baltic Sea food web—a mesocosm study. Can J Fish Aquat Sci 62:2240–2253

AOCS (American Oil Chemists' Society) (1997) Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. AOCS Official Method Ce 8–89

AOCS (American Oil Chemists' Society) (1998a) Fatty acid composition by GLC. Marine oils. AOCS Official Method Ce 1b–89

AOCS (American Oil Chemists' Society) (1998b) Preparation of methyl esters of long-chain fatty acids. AOCS Official Method Ce 2–66

Bagge O, Thurow F, Steffensen E, Bay J (1994) The Baltic cod. Dana 10:1–28

Baranova T (1995) The structure of spawning cod stock in the eastern Baltic during 1972–1995. ICES CM 1995/J:9: 1–6. ICES, Copenhagen

Bell JG, McEvoy J, Tocher DR, Sargent JR (2000) Depletion of alpha-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. J Nutr 130:1800–1808

Bleil M, Oeberst R (1997) The timing of the reproduction of cod (*Gadus morhua morhua*) in the western Baltic and adjacent areas. ICES CM DD 03:1–27. ICES, Copenhagen

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917

Brown NP, Shields RJ, Bromage NR (2006) The influence of water temperature on spawning patterns and egg quality in the Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 261:993–1002

Casini M, Cardinale M, Arrhenius F (2004) Feeding preferences of herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) in the southern Baltic Sea. ICES J Mar Sci 61: 1267–1277

Casini M, Cardinale M, Hjelm J (2006) Inter-annual variation in herring, *Clupea harengus*, and sprat, *Sprattus sprattus*, condition in the central Baltic Sea: What gives the tune? Oikos 112:638–650

Casini M, Bartolino V, Molinero JC, Kornilovs G (2010) Linking fisheries, trophic interactions and climate: threshold dynamics drive herring *Clupea harengus* growth in the central Baltic Sea. Mar Ecol Prog Ser 413:241–252

Casini M, Kornilovs G, Cardinale M, Mollmann C and others (2011) Spatial and temporal density dependence regulates the condition of central Baltic Sea clupeids: compelling evidence using an extensive international acoustic survey. Popul Ecol 53:511–523

Cerda J, Carrillo M, Zanuy S, Ramos J (1994) Effect of food ration on estrogen and vitellogenin plasma levels, fecundity and larval survival in captive sea bass, *Dicentrarchus labrax*: preliminary observations. Aquat Living Resour 7:255–266

Czeczuga B (1976) Carotenoids content in some crustaceans from the Baltic Sea. Bull Acad Pol Sci Biol 24:349–353

Dalsgaard J, St John M (2004) Fatty acid biomarkers: validation of food web and trophic markers using ^{13}C-labelled fatty acids in juvenile sandeel (*Ammodytes tobianus*). Can J Fish Aquat Sci 61:1671–1680

Dijkman NA, Kromkamp JC (2006) Phospholipid-derived fatty acids as chemotaxonomic markers for phytoplankton: application for inferring phytoplankton composition. Mar Ecol Prog Ser 324:113–125

Eliassen JE, Vahl O (1982) Seasonal variations in biochemical composition and energy content of liver, gonad and muscle of mature and immature cod, *Gadus morhua* (L.) from Balsfjorden, northern Norway. J Fish Biol 20:707–716

Flinkman J, Aro E, Vuorinen I, Viitasalo M (1998) Changes in northern Baltic zooplankton and herring nutrition from 1980s to 1990s: top-down and bottom-up processes at work. Mar Ecol Prog Ser 165:127–136

Fraser AJ, Gamble JC, Sargent JR (1988) Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). Mar Biol 99:307–313

Goetz FW, Garczynski M (1997) The ovarian regulation of ovulation in teleost fish. Fish Physiol Biochem 17:33–38

Grung M, Svendsen YS, Liaaenjensen S (1993) The carotenoids of eggs of wild and farmed cod (*Gadus morhua*). Comp Biochem Physiol B 106:237–242

Hemre GI, Karlsen O, Eckhoff K, Tveit K, Mangor-Jensen A, Rosenlund G (2004) Effect of season, light regime and diet on muscle composition and selected quality parameters in farmed Atlantic cod, *Gadus morhua* L. Aquacult Res 35:683–697

ICES (International Council for the Exploration of the Sea) (2007) Report of the ICES/HELCOM working group on integrated assessments of the Baltic Sea (WGIAB). ICES CM 2007/BCC:04:1–71. ICES, Copenhagen

ICES (International Council for the Exploration of the Sea) (2010) Report of the Baltic Fisheries Assessment Working Group (WGBFAS), ICES CM 2010/ACOM: 10:1–613. ICES, Copenhagen

Iverson SJ (2009) Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Arts MT, Brett MT, Kainz M (eds) Lipids in aquatic ecosystems. Springer, New York, NY, p 281–307

Janas U, Wocial JA, Szaniawska A (2004) Seasonal and annual changes in the macrozoobenthic populations of the Gulf of Gdansk with respect to hypoxia and hydrogen sulphide. Oceanologia 46:85–102

Jangaard PM, Ackman RG, Sipos JC (1967) Seasonal changes in fatty acid composition of cod liver flesh roe and milt lipids. J Fish Res Board Can 24:613–627

Kaitaranta JK, Ackman RG (1981) Total lipids and lipid classes of fish roe. Comp Biochem Physiol B 69:725–729

Karasiova EM, Voss R, Eero M (2008) Long-term dynamics in eastern Baltic cod spawning time: from small scale reversible changes to a recent drastic shift. ICES CM 2008/J:03:1-20. ICES, Copenhagen

Kirsch PE, Iverson SJ, Bowen WD, Kerr SR, Ackman RG (1998) Dietary effects on the fatty acid signature of whole Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 55: 1378–1386

Kjesbu OS (1989) The spawning activity of cod, *Gadus morhua* L. J Fish Biol 34:195–206

Kjesbu OS (1994) Time of start of spawning in Atlantic cod (*Gadus morhua*) females in relation to vitellogenic oocyte diameter, temperature, fish length and condition. J Fish Biol 45:719–735

Kjesbu OS, Klungsoyr J, Kryvi H, Witthames PR, Greer Walker M (1991) Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. Can J Fish Aquat Sci 48:2333–2343

Kjesbu OS, Righton D, Kruger-Johnsen M, Thorsen A, Michalsen K, Fonn M, Witthames PR (2010) Thermal dynamics of ovarian maturation in Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 67:605–625

Köster FW, Möllmann C, Hinrichsen HH, Wieland K and others (2005) Baltic cod recruitment—the impact of climate variability on key processes. ICES J Mar Sci 62: 1408–1425

Koven W, Henderson R, Sargent J (1994) Lipid digestion in turbot (*Scopthalmus maximus*) II: Lipolysis in vitro of ^{14}C-labelled triacylglycerol, cholesterol ester and phosphatidylcholine by digesta from different segments of the digestive tract. Fish Physiol Biochem 13:275–283

Kraus G, Tomkiewicz J, Köster FW (2002) Egg production of Baltic cod (*Gadus morhua*) in relation to variable sex ratio, maturity, and fecundity. Can J Fish Aquat Sci 59: 1908–1920

Lambert Y, Dutil JD (1997) Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? Can J Fish Aquat Sci 54:104–112

Larsson PO (1994) Recent development of the cod stocks around Sweden and possible reproduction disturbances. Report from the Uppsala Workshop on Reproduction Disturbances in Fish, Uppsala, 20–22 Oct 1993, p 26–34

Lie O, Sandvin A, Waagbo R (1994) Transport of alpha-tocopherol in Atlantic salmon (*Salmo salar*) during vitellogenesis. Fish Physiol Biochem 13:241–247

Lister AL, Van der Kraak G (2008) An investigation into the role of prostaglandins in zebrafish oocyte maturation and ovulation. Gen Comp Endocrinol 159:46–57

MacKenzie B, St John MA, Wieland K (1996) Eastern Baltic cod: Perspectives from existing data on processes affect-

ing growth and survival of eggs and larvae. Mar Ecol Prog Ser 134:265–281

Marshall CT, Yaragina NA, Lambert Y, Kjesbu OS (1999) Total lipid energy as a proxy for total egg production by fish stocks. Nature 402:288–290

Marteinsdottir G, Björnsson H (1999) Time and duration of spawning of cod in Icelandic waters. ICES CM 1999:34. ICES, Copenhagen

Matsuno T (2001) Aquatic animal carotenoids. Fish Sci 67: 771–783

Matsuyama M, Fukuda T, Ikeura S, Nagahama Y, Matsuura S (1994) Spawning characteristics and steroid-hormone profiles in the wild female Japanese sardine *Sardinops melanostictus.* Fish Sci 60:703–706

Meier HEM, Feistel R, Piechura J, Arneborg L and others (2006) Ventilation of the Baltic Sea deep water: a brief review of present knowledge from observations and models. Oceanologia 48:133–164

Miki W (1991) Biological functions and activities of animal carotenoids. Pure Appl Chem 63:141–146

Miki W, Yamaguchi K, Konosu S (1982) Comparison of carotenoids in the ovaries of marine fish and shellfish. Comp Biochem Physiol B 71:7–11

Möllmann C, Köster FW (2002) Population dynamics of calanoid copepods and the implications of their predation by clupeid fish in the central Baltic Sea. J Plankton Res 24:959–977

Möllmann C, Kornilovs G, Fetter M, Köster FW (2004) Feeding ecology of central Baltic Sea herring and sprat. J Fish Biol 65:1563–1581

Möllmann C, Kornilovs G, Fetter M, Köster FW (2005) Climate, zooplankton, and pelagic fish growth in the central Baltic Sea. ICES J Mar Sci 62:1270–1280

Mustafa T, Srivastava KC (1989) Prostaglandins (eicosanoids) and their role in ectothermic organisms. Adv Comp Environ Physiol 5:157–207

Neuenfeldt S, Beyer JE (2003) Oxygen and salinity characteristics of predator–prey distributional overlaps shown by predatory Baltic cod during spawning. J Fish Biol 62: 168–183

Nie XP, Zie J, Haeubner N, Tallmark B, Snoeijs P (2011) Why Baltic herring and sprat are weak conduits for astaxanthin from zooplankton to piscivorous fish. Limnol Oceanogr 56:1155–1167

Olsen RE, Henderson RJ, Sountama J, Hemre GI, Ringo E, Melle W, Tocher DR (2004) Atlantic salmon, *Salmo salar*, utilizes wax ester-rich oil from *Calanus finmarchicus* effectively. Aquaculture 240:433–449

Peters J (2006) Lipids in key copepod species of the Baltic Sea and North Sea—implications for life cycles, trophodynamics and food quality. PhD thesis, University of Bremen

Peters J, Renz J, van Beusekom J, Boersma M, Hagen W (2006) Trophodynamics and seasonal cycle of the copepod *Pseudocalanus acuspes* in the central Baltic Sea (Bornholm Basin): evidence from lipid composition. Mar Biol 149:1417–1429

Pickova J, Larsson PO (1992) Rearing experiments with cod. Comparison between Baltic cod and Skagerrak coastal cod. ICES CM 1992/F:12. ICES, Copenhagen

Pickova J, Dutta PC, Larsson PO, Kiessling A (1997) Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (*Gadus morhua*) stocks. Can J Fish Aquat Sci 54: 2410–2416

Salze G, Tocher DR, Roy WJ, Robertson DA (2005) Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. Aquacult Res 36:1488–1499

Sargent JR, Falk-Petersen S (1988) The lipid biochemistry of calanoid copepods. Hydrobiologia 167–168:101–114

Sargent JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SCM (eds) The biological chemistry of marine copepods, Vol 1. Clarendon Press, Oxford, p 59–108

Sargent J, McEvoy L, Estévez A, Bell G, Bell M, Henderson J, Tocher D (1999a) Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217–229

Sargent JR, Bell JG, McEvoy L, Tocher DR, Estévez A (1999b) Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177:191–199

Sargent JR, Tocher DR, Bell JG (2002) The lipids. In: Halver JE, Hardy RW (eds) Fish nutrition, 3rd edn. Academic Press, San Diego, CA, p 181–257

Shatunovskiy MI (1971) Alterations in the qualitative composition of lipids in the organs and tissues of the Baltic cod (*Gadus morhua callarias* [L.]) as the gonads mature. J Ichthyol 11:790–798

Sorbera LA, Asturiano JF, Carrillo M, Zanuy S (2001) Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). Biol Reprod 64:382–389

St John MA, Lund T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar Ecol Prog Ser 131: 75–85

Støttrup JG, Overton JL, Paulsen H, Möllmann C, Tomkiewicz J, Pedersen PB, Lauesen P (2008) Rationale for restocking the eastern Baltic cod stock. Rev Fish Sci 16:58–64

Syvaoja EL, Salminen K, Piironen V, Varo P, Kerojoki O, Koivistoinen P (1985) Tocopherols and tocotrienols in Finnish foods: fish and fish products. J Am Oil Chem Soc 62:1245–1248

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11:107–184

Tocher DR, Sargent JR (1984) Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. Lipids 19:492–499

Tocher DR, Zheng XZ, Schlechtriem C, Hastings N, Dick JR, Teale AJ (2006) Highly unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and nutritional regulation of fatty acyl 6 desaturase of Atlantic cod (*Gadus morhua* L.). Lipids 41:1003–1016

Tomkiewicz J, Köster FW (1999) Maturation process and spawning time of cod in the Bornholm Basin of the Baltic Sea: preliminary results. ICES CM Y 25:1–11. ICES, Copenhagen

Tomkiewicz J, Kraus G, Köster FW (2005) Timing of Baltic cod spawning and spawner demography. ICES CM: Q:23. ICES, Copenhagen

Tomkiewicz J, Tybjerg L, Jespersen A (2003) Micro- and macroscopic characteristics to stage gonadal maturation of female Baltic cod. J Fish Biol 62:253–275

Tomkiewicz J, Støttrup JG, Jacobsen C, Røjbek MC, Köster F (2009) Influence of lipids and fatty acid composition on Baltic cod (*Gadus morhua* L.) maturation and timing of spawning. ICES Council Meeting, Berlin, 2009, p 1–4

Uzars D (1994) Feeding of cod (*Gadus morhua callarias* L.) in the Central Baltic in relation to environmental changes.

Proc Symp Cod and Climate Change, Reykjavik, Aug 23–27 1993, ICES Mar Sci Symp 198. ICES, Copenhagen, p 612–623

Viso AC, Marty JC (1993) Fatty acids from 28 marine microalgae. Phytochemistry 34:1521–1533

Wasmund N, Uhlig S (2003) Phytoplankton trends in the Baltic Sea. ICES J Mar Sci 60:177–186

Wasmund N, Tuimala J, Suikkanen S, Vandepitte L, Kraberg A (2011) Long-term trends in phytoplankton composition in the western and central Baltic Sea. J Mar Syst 87:145–159

Weber JM, Brichon G, Zwingelstein G (2003) Fatty acid metabolism in rainbow trout (*Oncorhynchus mykiss*) tissues: differential incorporation of palmitate and oleate. Can J Fish Aquat Sci 60:1281–1288

Wiegand MD (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. Rev Fish Biol Fish 6: 259–286

Wieland K, Jarre-Teichmann A, Horbowa K (2000) Changes in the time of spawning of the Baltic cod: possible causes and implications for recruitment. ICES J Mar Sci 57: 452–464

Wright PJ, Trippel EA (2009) Fishery-induced demographic changes in the timing of spawning: consequences for reproductive success. Fish Fish 10:283–304

Ytrestoyl T, Bjerkeng B (2007) Dose response in uptake and deposition of intraperitoneally administered astaxanthin in Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua* L.). Aquaculture 263:179–191

Zalachowski W (1985) Amount and composition of food of cod (*Gadus morhua*) in the Southern Baltic in 1977–1982. Acta Ichthyol Piscat 15:95–117

Zillen L, Conley DJ, Andren T, Andren E, Bjorck S (2008) Past occurrences of hypoxia in the Baltic Sea and the role of climate variability, environmental change and human impact. Earth Sci Rev 91:77–92

Appendix 1. Fatty acid composition (% of total fatty acids in upper part of table, mg g^{-1} of wet wt in lower part of table) and fatty acid ratios of triacylglycerols (TAG) in livers from Baltic cod in different months in 2009. Values are means ± SD

% of fatty acids	Feb	Mar	May	Jul	Aug
14:0	2.7 ± 0.5	2.1 ± 0.5	2.6 ± 0.3	2.4 ± 0.6	2.4 ± 0.4
16:0	13.5 ± 0.9	13.1 ± 0.8	13.7 ± 1.2	13.7 ± 1.4	13.2 ± 0.6
18:0	2.6 ± 0.5	3.3 ± 0.5	2.4 ± 0.4	2.6 ± 0.5	2.7 ± 0.4
SFAs	19.8 ± 1.0	19.4 ± 1.2	19.6 ± 1.7	19.5 ± 1.8	19.0 ± 1.0
16:1n-7	5.1 ± 0.8	5.2 ± 1.1	4.4 ± 0.2	4.7 ± 0.3	4.7 ± 0.7
18:1n-9	19.7 ± 2.7	22.2 ± 1.9	23.5 ± 4.3	24.2 ± 4.7	22.9 ± 4.0
18:1n-7	3.9 ± 0.9	4.1 ± 0.8	3.1 ± 0.3	3.8 ± 0.5	3.7 ± 0.7
20:1n-9	1.5 ± 1.5	1.2 ± 0.4	0.8 ± 0.1	0.9 ± 0.2	1.0 ± 0.5
24:1n-9	0.6 ± 0.3	0.6 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
MUFAs	32.3 ± 2.9	34.4 ± 3.3	33.3 ± 4.1	35.2 ± 4.5	33.8 ± 3.7
16:2n-4	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
18:2n-6	2.9 ± 0.6	2.8 ± 0.7	3.3 ± 0.2	3.7 ± 0.7	4.0 ± 0.8
18:3n-3	2.2 ± 0.5	2.0 ± 0.7	2.3 ± 0.1	2.4 ± 0.2	2.5 ± 0.4
18:4n-3	1.8 ± 0.4	1.8 ± 0.5	1.6 ± 0.2	1.8 ± 0.4	1.9 ± 0.4
20:4n-6	1.0 ± 0.3	1.0 ± 0.2	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.2
20:5n-3	9.3 ± 1.9	9.1 ± 1.3	8.1 ± 0.7	7.6 ± 1.1	8.4 ± 0.6
22:5n-3	1.6 ± 0.6	1.4 ± 0.3	1.4 ± 0.4	1.4 ± 0.4	1.3 ± 0.5
22:6n-3	21.3 ± 3.0	20.0 ± 2.3	22.0 ± 4.6	20.0 ± 4.1	20.5 ± 4.0
PUFAs	43.3 ± 3.7	41.3 ± 3.2	42.7 ± 5.5	41.0 ± 5.4	42.6 ± 4.0
n-6	4.7 ± 0.4	4.7 ± 0.6	4.9 ± 0.2	5.3 ± 0.7	5.6 ± 0.6
n-3	38.1 ± 3.5	36.1 ± 2.9	37.3 ± 5.3	35.1 ± 5.5	36.5 ± 4.0
DHA/EPA	2.4 ± 0.7	2.3 ± 0.4	2.7 ± 0.5	2.6 ± 0.5	2.5 ± 0.5
EPA/ARA	10.1 ± 3.6	9.7 ± 2.6	11.1 ± 0.8	10.4 ± 2.0	12.6 ± 2.8
mg g^{-1}	Feb	Mar	May	Jul	Aug
14:0	9.3 ± 3.8	8.2 ± 2.6	9.8 ± 2.0	6.7 ± 1.9	10.7 ± 3.2
16:0	46.2 ± 19.3	51.1 ± 12.8	52.8 ± 13.3	37.9 ± 9.9	57.3 ± 13.3
18:0	8.8 ± 4.1	13.0 ± 5.0	9.4 ± 3.0	7.1 ± 1.8	11.9 ± 3.7
SFAs	67.2 ± 27.4	75.8 ± 19.9	75.2 ± 18.8	53.6 ± 13.6	82.9 ± 20.3
16:1n-7	16.8 ± 6.1	20.4 ± 6.6	16.9 ± 3.9	13.1 ± 3.5	20.1 ± 3.9
18:1n-9	70.4 ± 37.0	87.3 ± 25.0	91.8 ± 31.1	68.7 ± 26.5	101.8 ± 35.8
18:1n-7	12.5 ± 4.5	15.9 ± 4.7	11.9 ± 2.5	10.3 ± 2.6	15.7 ± 3.3
20:1n-9	4.5 ± 4.1	4.7 ± 1.6	3.1 ± 0.6	2.6 ± 0.8	4.1 ± 1.3
24:1n-9	1.9 ± 1.0	2.3 ± 0.9	2.7 ± 0.6	2.0 ± 0.3	2.9 ± 0.7

Appendix 1 (continued)

mg g^{-1}	Feb	Mar	May	Jul	Aug
MUFAs	110.1 ± 47.4	134.6 ± 36.6	129.1 ± 37.9	98.6 ± 33.6	148.1 ± 41.8
16:2n-4	1.8 ± 0.9	2.1 ± 0.6	2.3 ± 0.6	1.7 ± 0.2	2.3 ± 0.6
18:2n-6	10.3 ± 5.5	11.2 ± 4.0	12.6 ± 2.8	10.3 ± 2.9	17.5 ± 5.6
18:3n-3	8.0 ± 4.3	7.8 ± 3.7	8.9 ± 2.3	6.7 ± 2.0	11.0 ± 3.3
18:4n-3	6.3 ± 3.0	7.1 ± 3.1	6.2 ± 1.6	4.9 ± 1.8	8.2 ± 2.5
20:4n-6	3.2 ± 1.2	3.8 ± 1.1	2.8 ± 0.7	2.0 ± 0.5	3.0 ± 0.6
20:5n-3	33.1 ± 15.8	35.7 ± 11.0	31.5 ± 9.6	21.5 ± 8.0	36.4 ± 8.1
22:5n-3	4.7 ± 1.1	5.5 ± 1.6	5.3 ± 1.6	3.7 ± 0.9	5.5 ± 1.4
22:6n-3	73.6 ± 33.2	78.1 ± 20.0	84.4 ± 24.5	54.2 ± 14.4	86.3 ± 9.6
PUFAs	150.8 ± 67.0	162.3 ± 42.4	164.5 ± 43.5	112.7 ± 30.4	182.4 ± 28.9
n-6	16.4 ± 7.4	18.6 ± 4.9	18.6 ± 4.2	14.6 ± 3.7	24.3 ± 6.3
n-3	132.6 ± 58.9	141.6 ± 37.3	143.5 ± 39.0	96.4 ± 27.4	155.8 ± 22.5
DHA/EPA	2.4 ± 0.7	2.3 ± 0.4	2.7 ± 0.5	2.6 ± 0.5	2.5 ± 0.5
EPA/ARA	10.1 ± 3.6	9.7 ± 2.6	11.1 ± 0.8	10.4 ± 2.0	12.6 ± 2.8

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