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**INFLUENCE OF LIPIDS AND FATTY ACID COMPOSITION ON BALTIC COD (*GADUS MORHUA* L.) MATURATION AND TIMING OF SPAWNING**

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The Baltic Sea experienced an ecosystem regime shift in the late 1980ies with great changes in food web dynamics and a decrease of the Eastern Baltic cod stock biomass to a historically low level in 1992. This decline was caused by high fishing pressure combined with a recruitment failure (Köster et al. 2003; Möllmann et al. 2008). In addition, the main spawning time of Eastern Baltic cod shifted from April-June to July-August in the early 1990ies. Effects of temperature and demographic changes in the cod stock do not fully explain this shift (Wieland et al. 2000). However, also the prey composition and condition of sprat and herring being the main prey of Baltic cod changed during this period influencing cod dietary lipid intake. As cod uses liver lipid stores for reproduction changed lipid intake or deficiency of essential fatty acids (EFA) could influence ovarian development and thereby the spawning time.

Apart from energy, fatty acids (FA) and especially polyunsaturated fatty acids (PUFA) are required for normal growth and development including reproduction in fish (Tocher 2003). The most important PUFA in relation to reproduction are docosahexaenoic acid (22:6n-3 = DHA), eicosapentaenoic acid (20:5n-3 = EPA) and arachidonic acid (20:4n-6 = ARA). These PUFAs have a general role to help maintaining the structural and functional integrity of cells. ARA has furthermore a specific role as precursor of eicosanoids. Eicosanoids are paracrine hormones produced by virtually every tissue in the body and include prostaglandins (PG). These hormones have a wide range of physiological actions including effects on sexual maturation and reproduction (Sargent et al. 2002).

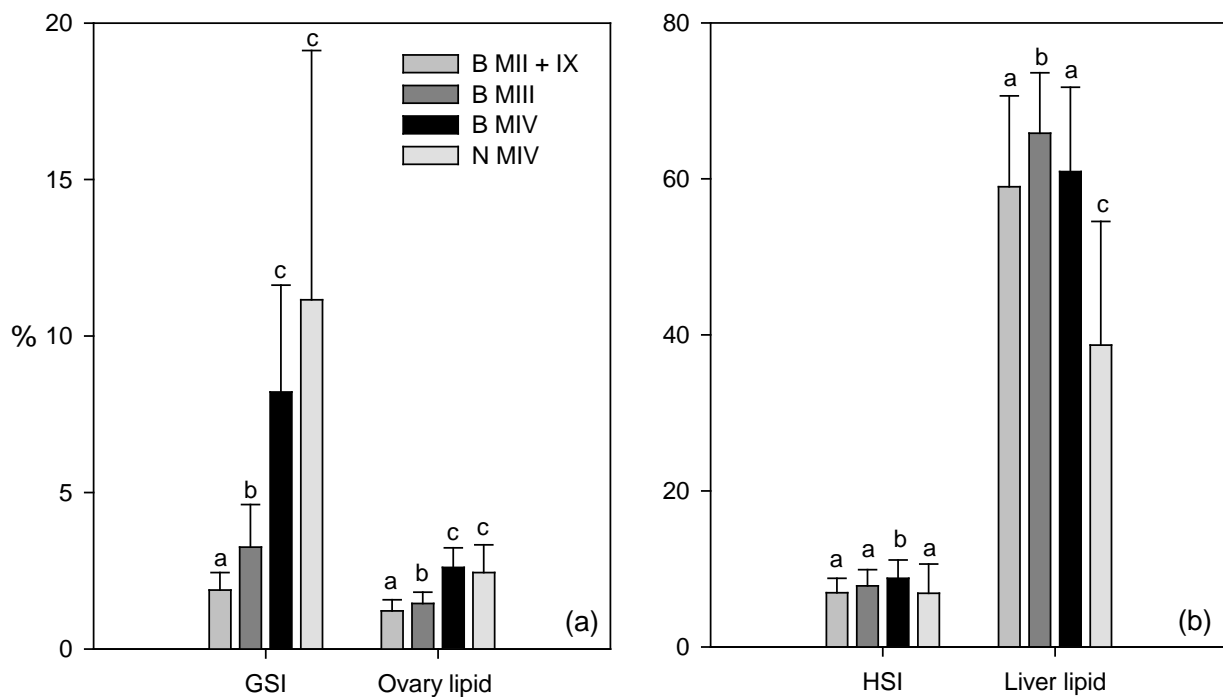
### **Materials and methods**

This study investigated the lipid content and fatty acid composition (FAC) in ovaries and liver of maturing female Baltic cod in different maturity classes and ovaries and liver of North Sea cod in prespawning condition for comparison. Cod were sampled on five research cruises conducted from November 2003 to April 2004 in the central Baltic Sea (ICES Sub-division 25) and two cruises in the North Sea in January 2004. In total, 231 females were sampled and morphometric data obtained. A sub-sample of ovarian tissue was preserved in formalin and analysed histologically. Liver and ovarian tissue was frozen until analysis of FAC using fatty acid methyl esters (FAME) and gas chromatography. Lipids from gonad and liver extracts were separated in phospholipids (PL) and neutral lipids (NL). Ovarian maturation was divided into immature (MII) and regeneration/resting stage (MIX) with no signs of reproductive development; maturation (MIII) was subdivided in FAC analyses depending on the most advanced oocytes: cortical alveoli = MIII<sup>0</sup>; peripheral yolk granules = MIII<sup>1</sup>; and yolk granules filling most but not the entire cytoplasm = MIII<sup>2</sup>; and late ripening stage, MIV, with significantly enlarged oocytes where yolk granules fill the entire cytoplasm

(Tomkiewicz et al. 2003). Multivariate data analyses of FAC were performed using the Unscrambler® v9.1 (CAMO, Oslo, Norway) software.

## Results and conclusions

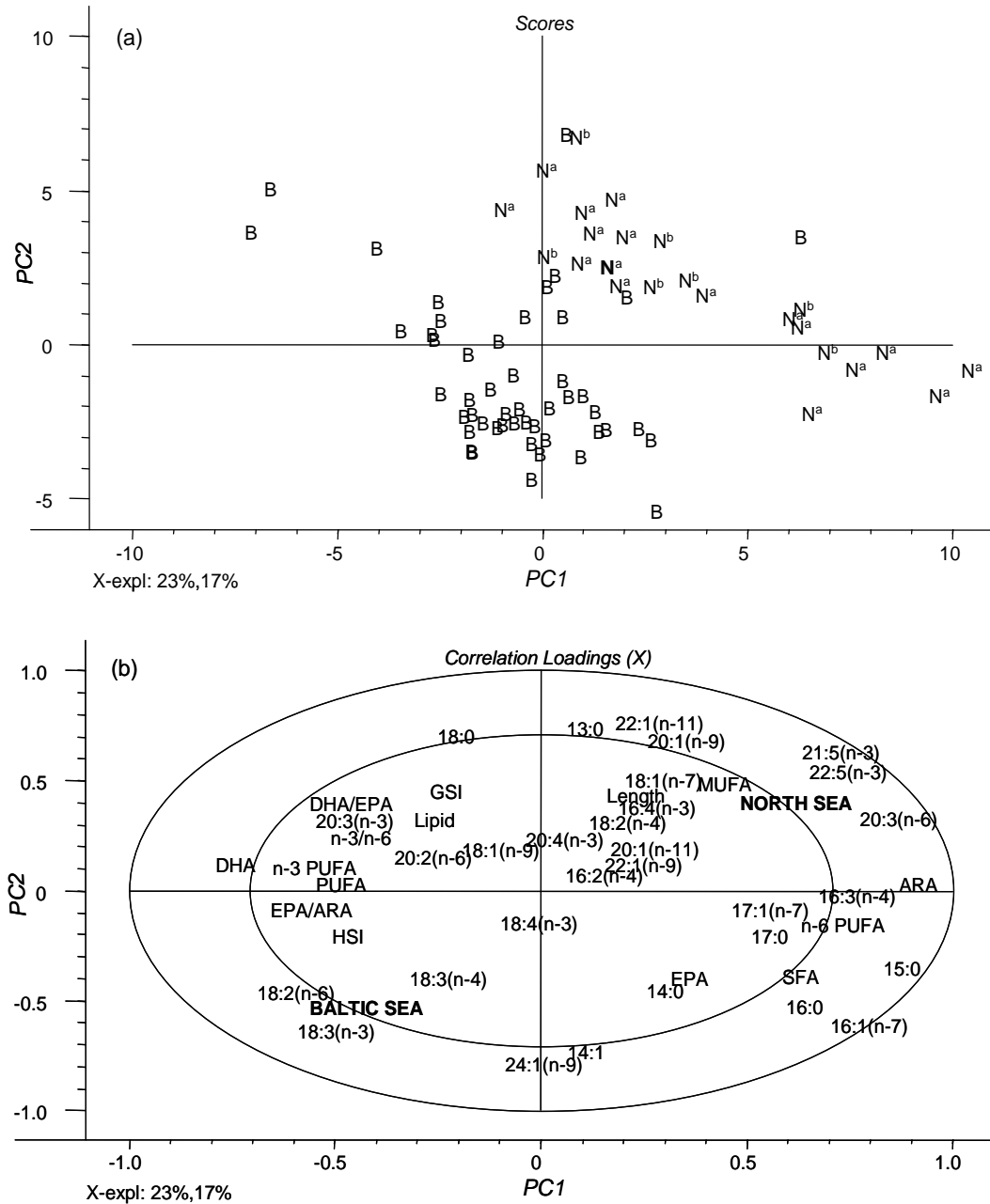
Total lipid increased significantly with increasing gonadosomatic index (GSI) in developing Baltic cod ovaries whereas the lipid content in prespawning (MIV) cod from the Baltic Sea and the North Sea was similar (Fig. 1). The hepatosomatic index (HSI) and total liver lipid level was, however, significantly higher in prespawning cod from the Baltic Sea than in North Sea cod, which eliminated poor energy reserves as a cause of the shift in spawning time.



**Figure 1.** *Gadus morhua*. (a) Means ± SD of GSI (gonad/body weight\*100) and lipid content of ovaries and (b) Means ± SD of HSI (liver weight/body weight\*100) and lipid content of livers in cod from the Baltic Sea, B ( $n = 49$ ) and the North Sea, N ( $n = 25$ ) in relation to maturity stage MII+IX, MIII and MIV.

The FAC in ovary PL and NL analyses showed that proportion of n-3 PUFA in Baltic cod ovaries gradually increased with progressive ovarian and oocyte development from MII+IX through MIII<sup>0</sup>, MIII<sup>1</sup> MIII<sup>2</sup> to MIV, while the proportion of n-6 PUFA in particular arachidonic acid (ARA) decreased. The comparison between FAC in ovary NL and PL of North Sea and Baltic Sea cod in pre-spawning condition (MIV) showed a significant difference between cod from the two areas (Fig. 2a). Baltic Sea cod ovaries contained proportionally high levels of n-3 PUFA and the EPA/ARA ratio was high whereas North Sea cod ovaries had high levels of n-6 PUFAs. The proportion of ARA in the MIV females was significantly lower in Baltic than in North Sea cod (Fig. 2b) The high EPA:ARA ratio in MIV ovaries of Baltic cod may reduce the quantity and hamper efficacy of eicosanoids responsible for different functions such as stimulation of oocyte

development. In addition, the DHA level was high in Baltic cod ovaries (Fig. 2b) with the DHA content in ovary PL and NL and liver PL with increasing GSI during seasonal development. In contrast, the DHL level was constant in liver NL indicating a selective allocation of DHA to developing ovaries while the negative correlation among the ARA level and GSI might illustrate a deficiency.



**Figure 2.** *Gadus morhua*. PCA scores plot (a) and loadings plot (b) of FAC, origin, lipid content, fish length, HSI and GSI in ovary PL from cod in maturity stage MIV. B refers to Baltic cod and N to North Sea cod. N<sup>a</sup> refers to the northern and N<sup>b</sup> to the southern part of the North Sea.

Baltic cod thus accumulated large amounts of lipids in the liver shown by the association with HSI in MIV (Fig. 1 and 2b), while specific EFAs seemed to be selectively mobilised to ovarian tissue during maturation. We reason that the climatic driven ecosystem changes in the Baltic Sea affecting phyto- and zooplankton dynamics and influencing the nutritional status of clupeid prey, caused a changed diet of cod with EFA limitation thereby prolonging the gonadal maturation of cod. The prey of Baltic cod being rich in n-3 PUFA but limited in ARA thereby could cause a delayed spawning time.

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**Keywords:** Cod, *Gadus morhua*, Clupeids, Lipid dynamics, Fatty Acid Composition, Maturation, Spawning time, Baltic Sea

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