Influence of environmental conditions, population density, and prey type on the lipid content in Baltic Herring (*Clupea harengus membras*) from the northern Baltic Sea

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Abstract

Global climate change can affect the energy content of fish by altering their lipid physiology and consumption. We investigated the effects of different environmental stressors on the lipid content of the Baltic Herring (*Clupea harengus membras*) from spawning ground samples that were collected annually in the northern Baltic Sea. During 1987-2014, the average lipid content of herring muscle decreased from 5-6% (w.wt.) to 1.5% (w.wt.). Generalized linear mixed models (GLMMs) indicated that sea water salinity and the size of the herring stock explained best the declining trend of lipid content. We estimated that the amount of the lipid storage incorporated in the spawning stock decreased by approximately 45% during the study, with respective energy content decreases. Fatty acid composition analysis revealed that herring lipids contained a high proportion of EPA (20:5n-3) and DHA (22:6n-3), which likely originated from its main summertime prey, *Limnocalanus macrurus.* The results illustrate various climate change-induced processes leading to changes in the lipid content of the Baltic Herring and, consequently, to changes in the energy flows of the northern Baltic ecosystem.

Introduction

Lipids are a major fuel source in aquatic food webs and the main energy source for fish (Brett et al. 1997). At all phases of their life-cycle, lipids are involved in several fish-related physiological processes. Lipids also maintain the structure and function of fish bio-membranes and participate in the synthesis, transportation and metabolism of hormones and other vital compounds (Sheridan 1994; Tocher 2003). In pelagial food webs, fatty acids (FA) are synthesized by phytoplankton and transferred along the food chains to zooplankton and fish. Most fish species tend to accumulate lipids in their muscle tissues or internal organs to be used as an energy source during periods when food is scarce. These lipid stores are needed for the build-up and maturation of gonads and for migrations between the feeding and spawning grounds, and the availability of lipid constituents is crucial for successful reproduction. For instance, egg fertilization and normal development of embryos and larvae require polyunsaturated fatty acids (PUFA), which females provide (e.g. Sheridan 1994; Rainuzzo et al. 1997; Tocher 2003; Muir et al. 2014). Of the PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are of special importance, as most fish species cannot synthesize them in sufficient quantities and their concentrations in consumers are diet-dependent.

Global climate change may impact fish energetics, as it widely affects the synthesis and dynamics of lipids in food webs by altering the environmental conditions where lipids are produced, transferred and consumed (Kattner et al. 2007). As the lipid content in fish tissues depends on available food resources, the main impact from this changing environment is likely to proceed along the food chain as a bottom-up effect (Litzow et al. 2006). Changes in the prey community and fish diet and/or increasing food competition in the feeding area can therefore weaken the possibilities of fish to collect sufficient lipid reserves. On the other hand, energy fluxes in the fish body can change, when fish must use more energy to overcome the physiological stress caused by an unfavourable physical or chemical environment. In this respect, the warming effect of climate change is detrimental, because increasing temperature accelerates fish metabolic rates, and this depletes their lipid reserves faster than under normal conditions (Biro et al. 2004). Studies on the effects of climate change on fish lipids remain limited, but they report that changes have already taken place as well in lakes as in the oceanic environments (e.g. Shulman et al. 2005; Litzow et al. 2006; Todd et al. 2008; Neff et al. 2012).

In the Baltic Sea, climate-driven changes are already manifested through increasing precipitation in the catchment area, decreasing salinity in sea water and as more frequently occurring mild winters (BACC 2015). In the Bothnian Sea (Fig. 1), which is the low-saline northern basin of the Baltic Sea, a major effect on the pelagic ecosystem is occurring through the appearance of an increased abundance of the large-sized copepod *Limnocalanus macrurus* and as a simultaneous herring (*Clupea harengus membras*) stock growth (Postel et al. 2011; Lindegren et al. 2011). Herring is the dominant planktivorous fish in this sea area and an important commercial fishery target with annual landings of

about 100 000 tons. As an abundant species, having a relatively high content of lipid and PUFA in the muscle tissue (Linko et al. 1985; Rajasilta 1992*a*; Szlinder-Richert et al. 2010), herring contribute significantly to ecosystem energy cycles, and its lipids affect all species consuming it, including humans (Aro et al. 2000; Arts et al. 2001). Large-scale variations in the Baltic ecosystem have revealed the role that hydro-climatic factors and food competition plays in the regulation of growth, condition and recruitment of herring (Axenrot and Hansson 2003; Casini et al. 2010; 2011; Lindegren et al. 2011), suggesting that the same environmental factors could influence also the biochemical properties of this species.

In the present study, our primary objective was to examine the effect of salinity, overwintering temperature and size of the herring stock on Baltic Herring lipid content. The lipid data were collected during 1987–2006 from the Archipelago Sea (Fig. 1), where, beginning in the 1980s, the herring population was monitored with annual samplings on the spawning grounds (Rajasilta et al. 1999). In addition to routine measurements of fish, the muscle tissue lipid content was determined from spawning females (Rajasilta 1992*a*) providing a data set spanning 19 years. Declining salinity, high variation in winter temperature and increasing herring stock during this period offered an opportunity for assessing the influence of the major environmental factors and intraspecific food competition on herring lipid reserves. As the time-series ended in 2006, the data were supplemented with more recent lipid analyses from spawning and overwintering fish and with FA composition analyses.

In the study area, the earliest studies on fatty acids in herring-derived lipids date back to the 1970s (Linko et al. 1985), but how much the lipid quality varies on the interannual or decadal time scale remains unknown. In this respect, the lipids of *Limnocalanus* were of special interest in the present work, because the parallel trend of its abundance with that of the herring stock suggests a causative link between the two species (Lindegren et al. 2011; Rajasilta 2014). Contemporary samplings of *Limnocalanus* and herring (Mäkinen et al. 2017) and comparisons of their FA composition were therefore conducted in order to better understand the role that *Limnocalanus* plays in Bothnian Sea herring energetics and population dynamics.

Materials and methods

Herring and environmental conditions in the study area

During the reproductive cycle, herring migrate between the spawning grounds in the innermost Archipelago Sea and the feeding and overwintering areas in the Bothnian Sea and the outer archipelago (Parmanne 1990; Kääriä et al. 2001). The mean depth of Bothnian Sea is 66 meters, and the sea area is characterized by a low salinity; which, at the sea surface, declined from approximately 5.8 to 5.4 between 1980 and present (Fig. 2). The Bothnian Sea halocline is weak, and stratification is mainly controlled by temperature (Håkansson et al. 1996). In winter, the sea is usually ice-covered at

least partly, but the duration of the ice period varies from year to year. During the current study, the maximum extent of the ice cover in the Baltic Sea varied between 49 000 km^2 (2007/08) and 405 000 km^2 (1986/87), but mild winters with reduced ice cover (less than the average 173 975 km^2) have become more frequent during the past years (Fig. 2; the Finnish Meteorological Institute).

In the Archipelago Sea, the spawning of herring starts usually in early May when the first shoals arrive on the spawning grounds, and ends in the middle of July, when the last shoals have reproduced (Rajasilta 1992b). The spawning shoals consist of fish of different lengths and ages (Rajasilta et al. 1993), but during the study, the mean length of the spawning population has decreased due to the reduction of growth rate (e.g. Rajasilta et al. 2015) (Fig. 3). The assessment reports from the feeding areas in the Bothnian Sea (=ICES subdivision 30) indicate approximately 160% increase in the stock size (Fig. 3), probably because of low predation pressure and/or increased abundance of prey organisms (Lindegren et al. 2011; Postel et al. 2011).

Sampling and treatment of fish

Spawning herring sampling for the lipid analyses was conducted annually in the Archipelago Sea (N60°23' E22°06') from 1987 to 2006 and continued in 2013–2014 (Table 1). The first lipid analyses were made from both sexes of spawning and overwintering herring (Rajasilta 1992*a*), but the monitoring was focused on spawning females due to the association between their lipid content and offspring production (Laine and Rajasilta 1999). Samples were taken from early spawning fish, as, in the study area, the muscle tissue lipid content differs between early spawning (May–middle of June) and late spawning herring (at the end of June and onwards) (Rajasilta 1992*a*). This is due to a dissimilar timing of feeding: early spawning fish collect their lipid reserves during the previous summer and autumn, whereas those spawning later are able to supplement their energy reserves during the spawning season due to the increase of zooplankton production (Rajasilta 1992*a*; 1992*b*; Rajasilta et al. 2001). Therefore, the winter temperature effect was expected to be greater in early spawning fish than in those spawning later in the season.

Samples consisting of 100–200 fish were collected from trap nets, which are used by the commercial fishery on the spawning grounds to catch herring. Fish were first examined for their sex and total length (1 mm precision), and gonad developmental stage was determined visually using the classification of Kesteven (Bagenal and Braum 1971): stages: 1-2 = resting, 3–4 = developing, 5 = ripe, 6 = spawning, 7 = spent). Then, a sample of ripe or spawning females was taken randomly out of this larger sample to determine the muscle tissue lipid content (Table 1). Fish were wrapped individually in aluminium foil and stored in plastic bags at -20°C until the analyses.

In addition to spawning herring, the lipid reserves of overwintering fish were investigated from a sample collected on January 3, 2017 in the same area as in 1988 (Rajasilta 1992*a*). Fish were taken

147 from the catch of a commercial trawler and measured as above. The mesenteric fat amount was
148 estimated visually using a relative scale (0 = no fat; 1 = fat distinguishable as a narrow thread along
149 the gut; 2–4 = increasing fat deposits in the body cavity). Only females were taken for the analyses of
150 lipid content, whereas mesenteric fat was examined from both sexes to be comparable with the earlier
151 data.

152 For the FA analysis, samples were collected in 2013 from the feeding area in the southern Bothnian
153 Sea (June 3 and September 26) and from the Archipelago Sea (June 17). In the Bothnian Sea, fish
154 were obtained from commercial trawlers catching herring in the open sea, and spawning fish were
155 taken from the trap net catch. Fish were brought into the laboratory in an insulated box having ice and
156 immediately measured for total length (1 mm precision), and their sex and maturity stage were
157 determined as above. Fish were gutted and carefully filleted and skinned, and the fillets were wrapped
158 individually in aluminium foil and stored at -80°C. The stomachs of fish were preserved in a 10%
159 formalin solution for the stomach content analysis, which was done using a dissecting microscope.
160 Only females (n = 5 at each date) were taken for the FA analysis, but the stomach contents were
161 studied from mixed samples (n = 10–24).

162 ***Analysis of the muscle lipid content***

163 The frozen fish samples were allowed to thaw, the skin was carefully removed and from each female,
164 a piece (2–5 g) of the dorsal muscle was dissected between the head and dorsal fin. The pieces were
165 weighed to the nearest 0.1 mg and dried in a freeze-dryer until they were fully dry and had a constant
166 weight. The dried samples were weighed to a 0.1 mg precision, homogenized and mixed with *ca* 0.5 g
167 anhydrous sodium sulphate to remove the moisture possibly absorbed by the tissue after drying. The
168 homogenate was extracted for six hours with diethyl ether in a Soxhlet apparatus. This procedure was
169 applied to ensure an efficient extraction of storage lipids (Castera et al. 1995). The solvent was
170 evaporated, and the lipid residue was weighed (to 0.1 mg precision). Lipid content was expressed as a
171 percentage of the sample's wet weight (% w.wt.).

172 ***Fatty acid analysis***

173 FA were extracted from 1.0–2.1 g skinned fillets by a modified Folch method (Folch et al. 1957) as
174 follows: 20 mL of methanol was added to the sample, and the mixture was homogenized with Ultra
175 Turrax T25 homogenizer (Janke and Kunkel - IKA Labortechnik, Staufen, Germany) at 8000 rpm for
176 2 min. A 40 mL amount of chloroform was added, and the mixture was homogenized again (8000
177 rpm, 2 min). The sample was filtered by vacuum. A 60 mL amount of chloroform-methanol (2:1, v/v)
178 was added to the residue, which was then subsequently homogenized at 8000 rpm for 3 min and
179 filtered. Containers and the blade of the homogenizer were flushed with 30 mL of
180 chloroform/methanol (2:1, v/v), and the extract was filtered. All the filtrates were combined. To the

combined filtrate, 37.5 mL of 0.88% (w/v) potassium chloride solution was added. After mixing, the upper phase was removed. The lower phase was washed with 75 mL of 0.88% potassium chloride (w/v) / methanol (1:1, v/v), collected and evaporated to dryness in a rotary evaporator. The sample was weighed and dissolved in 2 mL of chloroform. Fatty acid methyl esters (FAME) were prepared at 92°C by boron trifluoride-catalyzed transesterification from the lipid extracts after the solvent was evaporated under nitrogen (Morrison and Smith 1964; Ågren et al. 1992). FAME (dissolved in hexane) were analysed by gas chromatography with flame ionization detection (GC-FID) (PerkinElmer AutoSystem, Norwalk, CT) using a DB-23 column (60 m x 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, Palo Alto, CA). FAME were identified with the 68D FAME mixture (Nu-Chek-Prep, Inc.). In our analytics with common fatty acid types, the method utilizing boron-trifluoride-catalyzed transesterification has proven to be robust and trustworthy. To avoid possible problems with isomerization of fatty acids, it is important that fresh reagent is used when preparing FAME.

Environmental data and number of herring

Seawater salinity data in the southern Bothnian Sea (Station SR5) were obtained from the ICES (International Council for the Exploration of the Sea; HELCOM data set), consisting of annual measurements taken at the sea surface. As the water temperature is not recorded in the Bothnian Sea year-around, we used monitoring data from the Archipelago Sea, where the water column temperature is measured at 10-day intervals over the year. For each year, the reading means at 20 m depth during January–April were used to describe the average herring overwintering temperature (Fig. 2). The herring total number in the Bothnian Sea (ICES subdivision 30) was used as a model variable describing the size of the herring stock instead of the total stock biomass (TSB). This number was calculated from the stock assessment report (ICES 2015) by dividing TSB with mean weight of herring at different ages and then summing up the age-class numbers.

Statistical analyses

Two sets of generalized linear mixed models (GLMMs) were constructed to test the muscle lipid content (% w.wt.) for the monotonic trend occurring during 1987–2006 (=*Model I*) and to evaluate which of the environmental and biological factors influenced this trend (=*Model II*). The GLMMs were implemented in SAS 9.3 with the procedure GLIMMIX (SAS Institute Inc. 2009). In both GLM models, the lipid content was used as the dependent variable. In *Model I*, the year was used as the explanatory variable. In *Model II*, the log10(x+1)-transformed value of total number of herring, Bothnian Sea mean surface salinity and mean January–April temperature in the Archipelago Sea were used as fixed explanatory variables. As in some years (Table 1), the lipid content had a significant positive correlation with fish total length (Spearman rank correlation test; $p<0.05$), the length was included in *Model II* as a random effect. Based on analyses of distributions and covariance structures

of each variable in SAS Enterprise Guide® 4.3, a "*BETA*" distribution with its default link function "*Logit"* was applied in both GLM-models. In all models, the denominator degrees of freedom were calculated by Satterthwhaite approximation and a default method, the restricted maximum likelihood, was used as the estimation technique (SAS Institute Inc. 2009). Based on autocorrelation and heteroscedasticity tests, there was no need to correct for either of these issues. Also, no correction for over-dispersion was needed.

Because GLIMMIX cannot deal with data having long gaps in the time-series, the mean lipid content in the samples (% w.wt.) and the predictors (total number of herring, salinity and temperature during January–April) were also analysed for all study years (1987–2006 and 2013–2014) using Pearson product-moment correlation analysis after controlling for the normality of the variables with a one-sample Kolmogorov-Smirnov test (SPSS 22.0 statistical software; www.ibm/com/spss_statistics). When necessary, log-transformation was added to correct for the skewness in the distribution. Selected pairwise comparisons were performed with a Student's t-test for normal variables and Mann-Whitney U-test for non-normal variables. The annual trend in the coefficient of variation (CV) of the lipid content was tested with Pearson product-moment correlation analysis (no autocorrelation observed).

One-way analysis of similarity (ANOSIM) (Clarke and Warwick 2001) was used to test for differences in single FAs and lipid species in the total lipid content (% of total FAs) in herring (HR) and in *Limnocalanus* (LM) during June 2013 (HR n = 10; LM n = 1 pooled sample consisting of 30 adults) and September 2013 (HR n = 5; LM n = 3 pooled samples, each consisting of 30–32 adults). The contribution of single FAs and lipid types to dissimilarities between the species were tested using similarity percentage analysis (SIMPER) with Bray-Curtis dissimilarities (Clarke 1993; Clarke and Gorley 2006). The analyses were done with untransformed FA data using the Vegan package (Oksanen et al. 2016) in the R statistical software (R Development Core Team 2015).

Results

The analysis using individual measurements of the lipid content (n = 340; Table 2) indicated a significant decrease in spawning female herring between 1987 and 2006 (GLM *Model I*; $p<0.001$; Table 2). During this period, the average values decreased from 6% to 1.7% (Fig. 4), and the strongest change took place between 1987 and the end of the 1990s. During the first study years, the difference between the minimum and maximum values was clearly larger than at the end of the time-series. For example, in 1988, the lipid content ranged between 1.9% and 11.7% in the sample, while in 2006, the minimum value was 0.1% and maximum only 3.5% (Fig. 4). Also, the results from more recent years (2013 and 2014) indicated low sample mean values (mean = 1.5% and 1.6%, respectively) and decreased maximum and minimum values (Fig. 4). However, during 1987–2006, the coefficient of

variation (CV; Fig. 4) was high throughout the data set suggesting a rising trend in the variability of the lipid content (Pearson's correlation test; $r = 0.53$; $p = 0.019$; $n = 19$).

The difference in the mean lipid content between the early (data from 1987–88 combined: mean ± SD = 5.4 ± 2.7% w.wt.) and late (2013–14 data combined: mean ± SD = 1.5 ± 0.9% w.wt.) study years was statistically significant (Student's t-test; $t = 8.718$; $p < 0.001$; $df = 54$) displaying an overall decrease by approximately 70% in the sample means. A decrease was observed also in the samples collected in winter from the outer archipelago (Fig. 4). In 1988, the lipid content of the muscle was on average 7.7% (SD = 4.5), but 4.3% (SD = 2.9) in winter 2017. Likewise, mesenteric fat amount was higher in 1988 (mean ± SD = 2.5 ± 0.9 on a relative scale of 0–4; $n = 46$) than in 2017, when 77% of examined fish had no mesenteric fat at all (mean ± SD = 0.2 ± 0.4; $n = 31$). The difference in both parameters was significant (Mann-Whitney U-test; lipid content: $p = 0.01$; mesenteric fat: $p < 0.001$).

Of the three variables fitted in the model (GLM *Model II;* individual measurements), both salinity and the total number of herring best explained the trend found in the lipid content ($p = 0.01$ and $p = 0.02$, respectively; Table 2). The bivariate correlation analysis using the annual means of lipid content from all study years (1987–2014) confirmed this result (Fig. 5) showing a negative and significant relationship with the total number of herring ($r = -0.63$; $p = 0.01$; $n = 21$) and a positive relationship with salinity ($r = 0.52$; $p = 0.05$; $n = 20$). Overwintering temperature, which varied between -0.2 and 2.0°C during the study years (Fig. 2), showed a negative relationship with the average lipid content, but this relationship was only indicative ($r = -0.38$; $p < 0.1$; $n = 21$; Fig. 5). However, the GLM model utilizing the individual measurements from the years 1987–2006 indicated that the effect of temperature was significant ($p = 0.03$; Table 2). The Pearson's correlation test gave similar results also when only the years 1987–2006 were analysed.

In total, during 2013, 16 major fatty acids were identified in herring lipids with the rest being present in trace amounts or at a very low concentration (group "Others" in Table 3). Saturated FA (SAFA) formed about 27% of the total FA at all sampling dates, and in this group, palmitic acid (16:0) was the most abundant single FA with relative proportions of 21–22%. In the samples, monounsaturated FA (MUFA) constituted 23–30% and PUFA 37–43% of the total FA (Table 3). In general, single FA displayed a relatively stable pattern among the samples with exception of oleic acid (18:1n-9), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Table 3). The DHA proportions showed the highest variation among the FA being on average 21.1% (SD = 6.9) in females collected from the feeding area before spawning (gonad stage 4–5), and 14.9% (SD = 3.4) in September, when fish were recovering (gonad stage 1-2).

Spawning herring stomachs ($n = 10$) were empty, and in September, fish ate mysid shrimps (*Mysis* spp.). In June, all fish collected from the Bothnian Sea before spawning ($n = 24$) had eaten *Limnocalanus*, which, in the fish stomachs examined for FA composition, was the only prey organism.

The herring lipids contained largely the same FA as those of *Limnocalanus* (Table 3), but their relative proportions were not equal. ANOSIM indicated differences between *Limnocalanus* and herring in the composition of single FA ($r = 0.95$; $p = 0.001$) and in the types of FA (SAFA, MUFA and PUFA), which were also separated by some degree of overlap ($r = 0.78$; $p = 0.001$). SIMPER analysis showed that 30.6% of the dissimilarity found between species was explained by the contribution of the following individual FA in decreasing order of importance: 16:0, "Others," 18:1(n-9), EPA, DHA, 16:1(n-7), and 18:2(n-6). Furthermore, 19.67% of the dissimilarity found between species was explained by the contribution of SAFA, MUFA and PUFA (Table 4).

Discussion

The lipid content of the Baltic Herring muscle fluctuates in an annual cycle, with maximum values at the end of the feeding period in winter and minimum values at the spawning time (Rajasilta 1992*a*; Szlinder-Richert et al 2010; Aro et al. 2000; RØjbek et al. 2014). Herring store lipids also in the mesenteries around the gut, but these depositions are likely used for gonadal production, as they are depleted during winter and spring when the gonads develop (Rajasilta 1992*a*). Therefore, in the current study, visceral fat was not found in the spawning fish monitored.

In the spawning population, the average muscle tissue lipid content decreased significantly during 1987–2006 and was at a low level also during 2013–14. Besides the annual trend, the lipid content was characterized by high maximum values, which, by the end of the 1990s, disappeared from the samples. A high variation of fish lipid content is a typical finding (e.g. Anthony et al. 2000; Lane et al. 2011), which may reflect different experience in the foraging performance or genetic differences in the efficiency of food intake and/or food conversion ratio (Reiriz et al. 1998; Silverstein 2002). The Baltic Herring lipid content is known to vary according to fishing grounds, fish size and maturation stage of gonads, besides the seasonal variation (Rajasilta 1992*a*; Szlinder-Richert et al. 2010). Here, fish size was considered as a random effect in the GLM models, and by focusing the monitoring on females having the same maturity status, gonadal stage effects were excluded. Moreover, the fish sampling from the same place and during the same spawning time reduced spatial and temporal factor effects that possibly influenced the lipid content. In all, the temporal changes in the sample means and the range of the lipid values come from an external source instead of the sampling procedure itself.

In the Bothnian Sea, spawning female lipid content was influenced by salinity, winter temperature and herring population size. In other studies, the association between herring condition and salinity exists (Rönkkönen et al. 2004; Casini et al. 2010, 2011), but in these prior reports, salinity is considered as a background factor that affects fish indirectly through changes in the prey community and fish diet. Yet, low salinity results in direct energy costs for marine species due to osmoregulation between blood

water and electrolytes. In the Bothnian Sea, salinity has decreased by approximately 10% from the highest values in the 1980s and this change may have caused the herring an increased need to use energy for osmoregulation. Depending on the species, osmoregulation may take 10–50% of the total energy budget of fish leaving less energy for growth and other physiological functions (Boeuf and Payan 2001 and references therein). The effects of salinity on adult Baltic Herring are poorly known, but in a laboratory experiment with herring juveniles, the lipid reserves clearly grew with increasing salinity (Rajasilta et al. 2011). Presuming a similar effect in adult herring, the decline of salinity could, therefore, have forced the fish to allocate more energy to osmoregulation, which may in turn have consumed their lipid reserves. In this case, the net energy level, measured as the lipid content, could have decreased even though food consumption was stable.

Moreover, during the study period, spawning female lipid content was influenced negatively by overwintering temperature, which varied between -0.2°C and +2.0°C. The negative association between temperature and lipid content suggested that energy consumption increased during mild winters. Increased energy consumption could be due to higher basic metabolic needs (e.g. Clarke and Fraser 2004) but also due to increased fish swimming activity. During mild winters, reduced ice cover allows greater light penetration, which may increase activity costs; increased light and temperature are known to positively influence Baltic Herring swimming behaviour (Didrikas and Hansson 2009).

Our results suggest that salinity and temperature decrease herring lipid content, potentially through increased catabolic processes, but the initial magnitude of lipid depositions is determined by the quantity and quality of food. In the Bothnian Sea, herring feed on mesozooplankton in summer and on mysids and amphipods during autumn (Flinkman et al. 1998; Rajasilta et al. 2014). However, the degree to which prey diversity has varied in the herring diet and in the environment remains unknown. In the Bothnian Sea, zooplankton monitoring conducted during August (Postel et al. 2011) indicates a decreasing trend in the biomass of some zooplankton species (e.g. *Acartia* spp.) and an increase of some others (e.g. *Eurytemora affinis*, *L. macrurus*, *Bosmina longispina maritima*), but the long-term variation of macroscopic prey, which herring feed on from September onwards, is not known. This is particularly true in the region of study during autumn when the lipid stores are formed (Aro et al. 2000).

In Atlantic and Pacific Herring, prolonged starvation leads to a reduction in their muscle lipid content (Wilkins 1967; Hay et al. 1988). The observed decrease in lipid content in our study suggests prolonged starvation especially in spawning fish, and the lipid content of overwintering herring supports this result. During the winter of 2017, most fish had no mesenteric fat in their body cavity at all, and the muscle lipid content was substantially lower in 2017 than in 1988, when the previous winter samples were analysed (Rajasilta 1992*a*). The sample average decreased from 7.7% to 4.3%

between 1988 and the present when estimated from the lipid content of the overwintering fish, and in the spawning herring, the difference was of a similar magnitude. Although salinity and temperature may contribute to this change by increasing the consumption of metabolic energy in fish, lipid content also depends on the food resources available in the environment. Thus, the negative effect of the herring population number on the lipid content may be partly due to intraspecific resource competition and decreased prey/fish ratio.

Herring prey are not evenly distributed but show scattered aggregations in the environment (Aschan 1988; Korpinen and Westerbom 2010; Klais et al. 2016). Under such circumstances, fish foraging success depends on their competitive abilities, because the aggregations of prey attract the fish increasing competitive interactions among the individuals (Ward et al. 2006). Decreasing availability of prey could explain the disappearance of the highest lipid values from the samples, because when prey organisms become scarce, not even the best competitors can find food and form rich lipid depositions. As shown by the coefficient of variation of the lipid content, between 1987 and 2006, the differences between individual fish were continuously high or even increased. This may indicate increased food competition in the herring population or between herring and some another species. For instance, in the Bothnian Sea at the end of the 1990s, in the commercial catches, sprat (*Sprattus sprattus*) appeared (statistics of the Natural Resources Institute Finland). Sprat does not reproduce in the Bothnian Sea due to low salinity, but its rapid stock growth in the Central Baltic in the 1990s caused its expansion northwards (Voss et al. 2011).

The increase in the Bothnian Sea herring stock may be one of the many climate change-induced consequences. In the Bothnian Sea, freshening water has increased the biomass of the glacial-relict copepod *Limnocalanus* (Postel et al. 2011), which provides a rich food supply for the herring in spring and early summer due to its large body size and high lipid content (Rajasilta et al. 2014; Mäkinen et al. 2017). Abundant and high-quality prey has most likely improved the survival of adult herring resulting in the population growth (Lindegren et al. 2011). Therefore, in the herring population during late summer and autumn, competition for food has increased, as from May to mid-June, *Limnocalanus* is available for the herring only during a short time period. During the beginning of July, adults and copepod stages move to deep- and cold-water layers where herring do not usually feed (Mäkinen et al. 2017), and other zooplankton prey may be too scarce to provide enough energy for the increased herring stock.

During the spawning period 2013, herring lipids contained about 37–43% of PUFA, which correspond to the values found in southern Baltic Sea herring (Szlinder-Richert et al. 2010). In our study area, the FA pattern seems to be relatively unchanged when comparing the results of the current study with those from 1976, when herring lipids were also investigated at the same dates and with similar

methods (Linko et al. 1985). The largest difference between data found between 1976 and 2013 was in the proportions of total PUFA, EPA and DHA, which all increased. The overall increase of PUFA was approximately 8–12% between 1976 and 2013 suggesting a long-term change in the trophic conditions and herring diet. Herring from other areas of the Baltic Sea demonstrate an increase of n-3 PUFA (Lind et al. 2018), which supports the results of the present study and indicates a general trend in the FA pattern of herring lipids.

The effect of *Limnocalanus* on herring lipids seems to be greatest in summer, because the SIMPER analysis indicated approximately 70% similarity in the proportions of single FAs between herring and *Limnocalanus* in 2013, and the similarity was even higher in total PUFA. A complete similarity could hardly be expected between the two species as fish fed also on mysids. Moreover, the fish FA composition can deviate from that of their prey due to the biochemical processes, which fish use to modify the dietary FAs further (Tocher 2003). However, lipogenesis does not explain the high proportions of total PUFA, EPA and DHA in the herring lipids, as evidence to date suggests that marine fish are not capable of synthesizing them (Tocher 2003). Thus, the result shows that the high proportion of essential FA (EFA) in the herring lipids was due to feeding on *Limnocalanus*, which most likely acts as a PUFA mediator from the spring phytoplankton bloom. The close relationship between the lipids of herring and *Limnocalanus* also suggests that the increase of PUFA in herring at spawning time, observed between 1976 and 2013, could be due to an increased abundance of *Limnocalanus*.

Potential implications for the herring stock and the food-webs in the Bothnian Sea

Even in small geographical areas, Neff et al. (2012) reported that the mechanisms behind the lipid trends are usually complex and dependent on the local conditions. In the current study, the results of the GLM model and correlation analyses were mostly consistent, indicating that sea water salinity, winter temperature and size of the herring stock have an impact on the herring energy content. The contribution of each of these variables to the lipid content could not be shown from the field samples, because they all act together and each of them could add, decrease or counteract the effect of the others thus modifying the result. In the Baltic Sea, where environmental conditions vary annually, the interplay of different variables provides the herring an opportunity to survive and reproduce as the lack of energy reserves in one year can be compensated for in another. The situation may be different in the future, if salinity, temperature and feeding conditions cause a negative effect on the lipid content year after year.

In the Baltic Sea, climate models predict a further decline of salinity and increasing winter temperatures (Vuorinen et al. 2015; BACC 2015), which suggests that the negative effect on the

herring lipid reserves is likely to continue. This may reduce the reproductive success of herring, as female lipid content is correlated positively with herring egg survival and hatching success (Laine and Rajasilta 1999). On the other hand, EFA content in female herring lipid plays a more important role in the reproductive success than lipid quantity alone (e.g. Pickova et al. 1997; Rainuzzo et al. 1997). In this respect, the Bothnian Sea environmental changes may have also caused positive effects on herring, as the abundance of high-quality prey has increased at the spawning time when, in females, the requirement of EFA is particularly high.

The most prominent sign of a change in the energy flows in the Baltic ecosystem has been reductions in herring growth rates (Casini et al. 2010; 2011; Rajasilta et al. 2015). With declining body size, herring follow the trend found across a wide range of species as a response to climate change (Gardner et al. 2011). Reduction of the body size is one way to diminish the costs of energy, but fish may adopt other means to conserve their resources. They can abandon their normal reproductive cycle and choose not to reproduce until the energy reserves are replenished (Rideout and Tomkiewicz 2011); or they save energy through behavioral adjustments, for example, by changing their migratory pattern. As the migrations between spawning and feeding areas consume plenty of energy (Slotte 1999; Varpe et al. 2005), fish could stay closer to the spawning grounds after spawning instead of migrating to the distant normal feeding areas. Change of migration routes can bring about detrimental effects in the food web, because it may disconnect the energy flow and transportation of fatty acids from one area to another (van Deurs et al. 2016).

The Baltic Herring transports lipids mainly in their muscle tissue, which forms on average 34% of the total body mass of a fish (Rajasilta et al. 2015). If our results reporting the herring lipid content are representative for Bothnian Sea herring overall, then at the end of the 1980s, the spawning stock contained approximately 137 474 tons muscle tissue with 6 870 tons of storage lipids (stock biomass according to ICES 2015). The total amount of muscle tissue increased with increasing stock size to approximately 236 615 tons in 2014, but in spite of this, the stock contained only about 3 780 tons lipids. This further indicates a large change in ecosystem energy cycles, where herring are an important prey for predatory fish, grey seals and seabirds. Because of the low lipid content of herring at present, species preying upon it need to eat more to acquire the same amount of energy as reported in the 1980s, especially as herring size has also decreased. Poor energy quality of fish may, for instance, decrease the conditions for fish-eating seabird offspring (Österblom et al. 2001), which inevitably leads to a higher mortality. However, the low lipid content might also cause positive effects as it may reduce the accumulation of lipophilic toxic compounds. High level of dioxins and PCB-compounds has limited the use of the Baltic Herring for human consumption, but recently their concentrations have decreased (Airaksinen et al. 2014), possibly also reflecting the decreased lipid content in addition to the decrease in the emissions.

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678 **Figure legends**

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680 **Figure 1** The study area in the northern Baltic Sea. Samples of spawning herring were collected in the
681 Archipelago Sea (AS; filled circle indicates the sampling location) and trawl samples in the Bothnian
682 Sea (BS= the ICES subdivision 30) and the outer archipelago.

683 **Figure 2** Upper panel: average salinity in the Bothnian Sea and temperature in the Archipelago Sea for
684 January–April at 20 m depth and 2-year moving average (lines) in 1976–2014 (grey bold line indicates
685 the study period). Lower panel: Severity of winters in the Baltic Sea in 1976–2015 expressed as
686 anomalies from the average extent of permanent ice cover (mean= 173 975 km^2); (Data sources: Ice
687 cover statistics/Finnish Meteorological Institute; SW salinity/ ICES Dataset on Ocean Hydrography;
688 SW temperature/own data).

689 **Figure 3** Mean length (cm) and age (years) of herring in the spawning population of the study area
690 and total number of herring in the Bothnian Sea (Nr x 10^{10}) during 1984-2014. (Herring abundance
691 according to ICES (2015)).

692 **Figure 4** (a) Lipid content (% w.wt.) of the muscle tissue in spawning Baltic Herring females in the
693 Archipelago Sea in 1987–2006 and 2013–14, and (b) lipid content (% w.wt.) and amount of
694 mesenteric fat (relative scale from 0 to 4) in herring samples collected in winter from the Archipelago
695 Sea in 1988 and 2017. In the large panel, bold solid line indicates the sample means with standard
696 deviation (vertical bars), dashed lines the maximum and minimum values in the samples and grey line
697 the coefficient of variation (CV). In the small panel, values of *p* indicate the significance level in
698 comparisons between 1988 and 2017 (Mann-Whitney U test). ND = no samples were obtained.

699 **Figure 5** Relationship between the lipid content of the muscle tissue in spawning Baltic Herring
700 females and (a) total number of herring in the Bothnian Sea; (b) mean SW salinity and (c) mean SW
701 temperature in January-April; time series from 1987-2006 and 2013-14.

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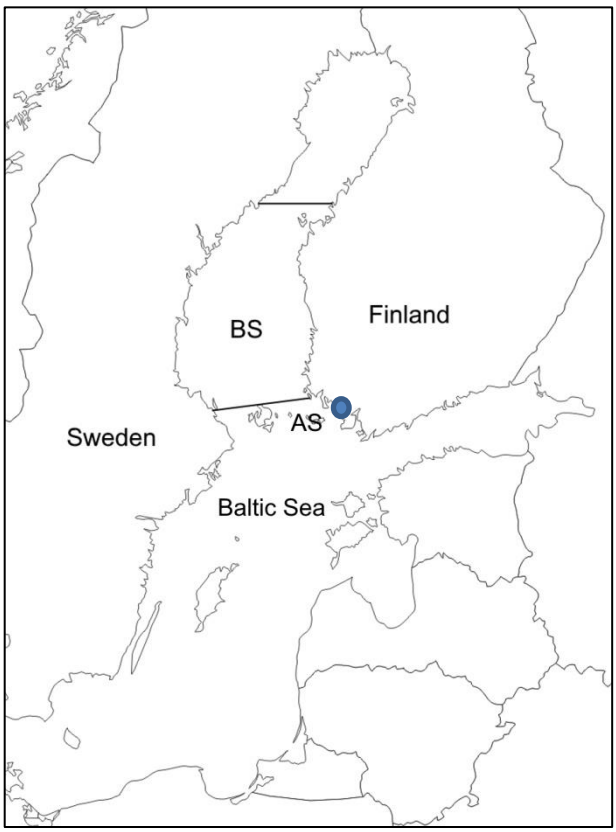

Figure 1 The study area in the northern Baltic Sea. Samples of spawning herring were collected in the Archipelago Sea (AS; filled circle indicates the sampling location) and trawl samples in the Bothnian Sea (BS= the ICES subdivision 30) and the outer archipelago.

Fig. 2

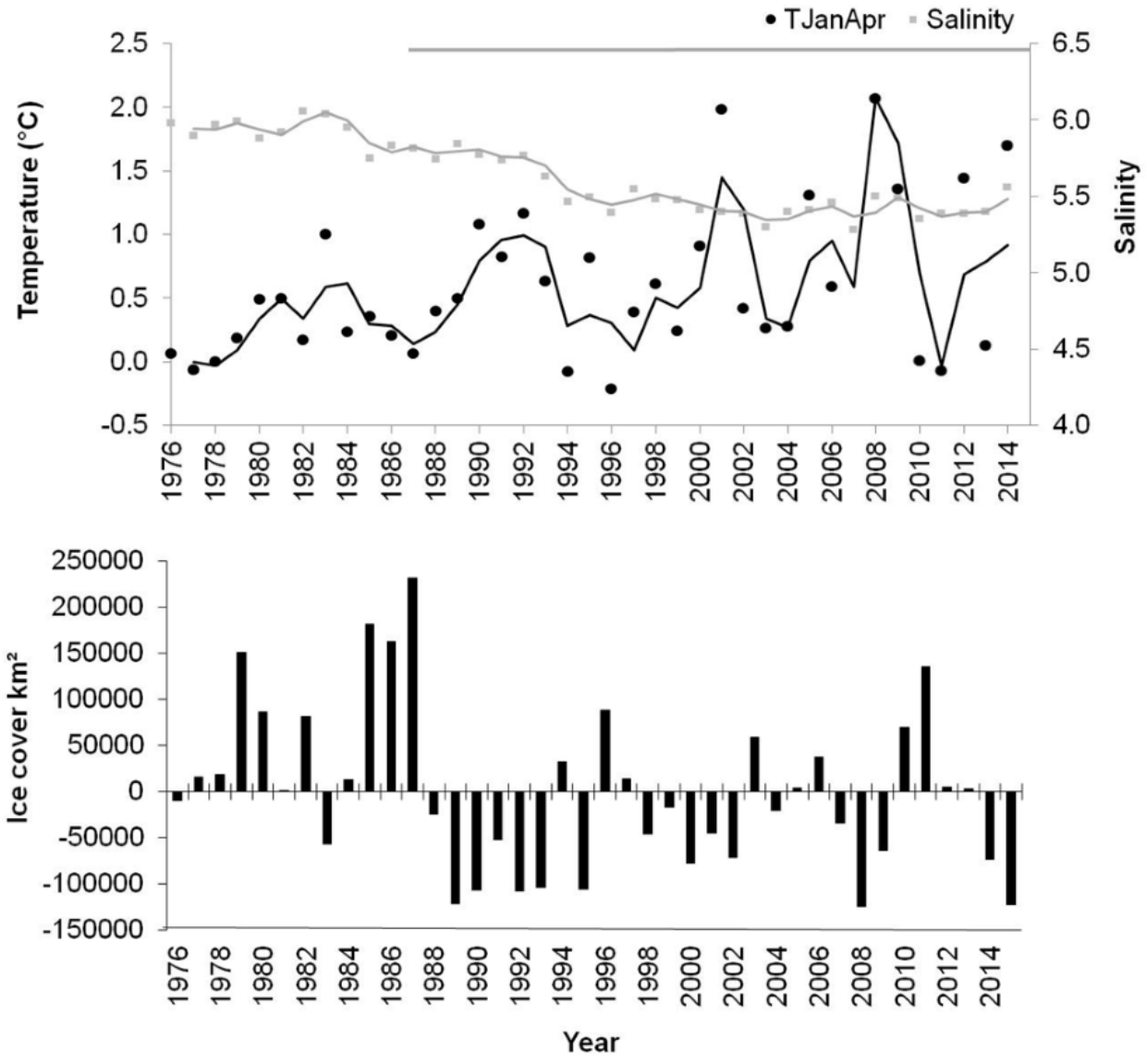

Figure 2 Upper panel: average salinity in the Bothnian Sea and temperature in the Archipelago Sea for January–April at 20 m depth and 2-year moving average (lines) in 1976–2014 (grey bold line indicates the study period). Lower panel: Severity of winters in the Baltic Sea in 1976–2015 expressed as anomalies from the average extent of permanent ice cover (mean= 173 975 km^2); (Data sources: Ice cover statistics/Finnish Meteorological Institute; SW salinity/ ICES Dataset on Ocean Hydrography; SW temperature/own data).

Fig. 3

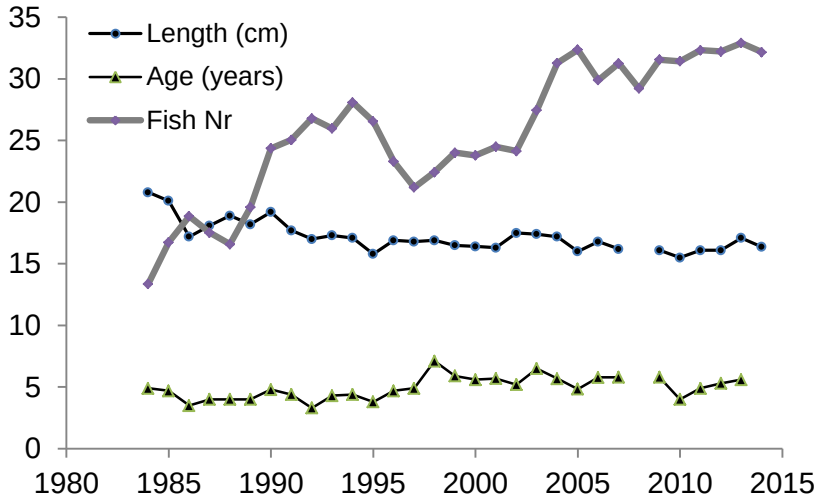

Figure 3 Mean length (cm) and age (years) of herring in the spawning population of the study area and total number of herring in the Bothnian Sea (Nr x 10^{10}) during 1984-2014. (Herring abundance according to ICES (2015)).

Fig. 4

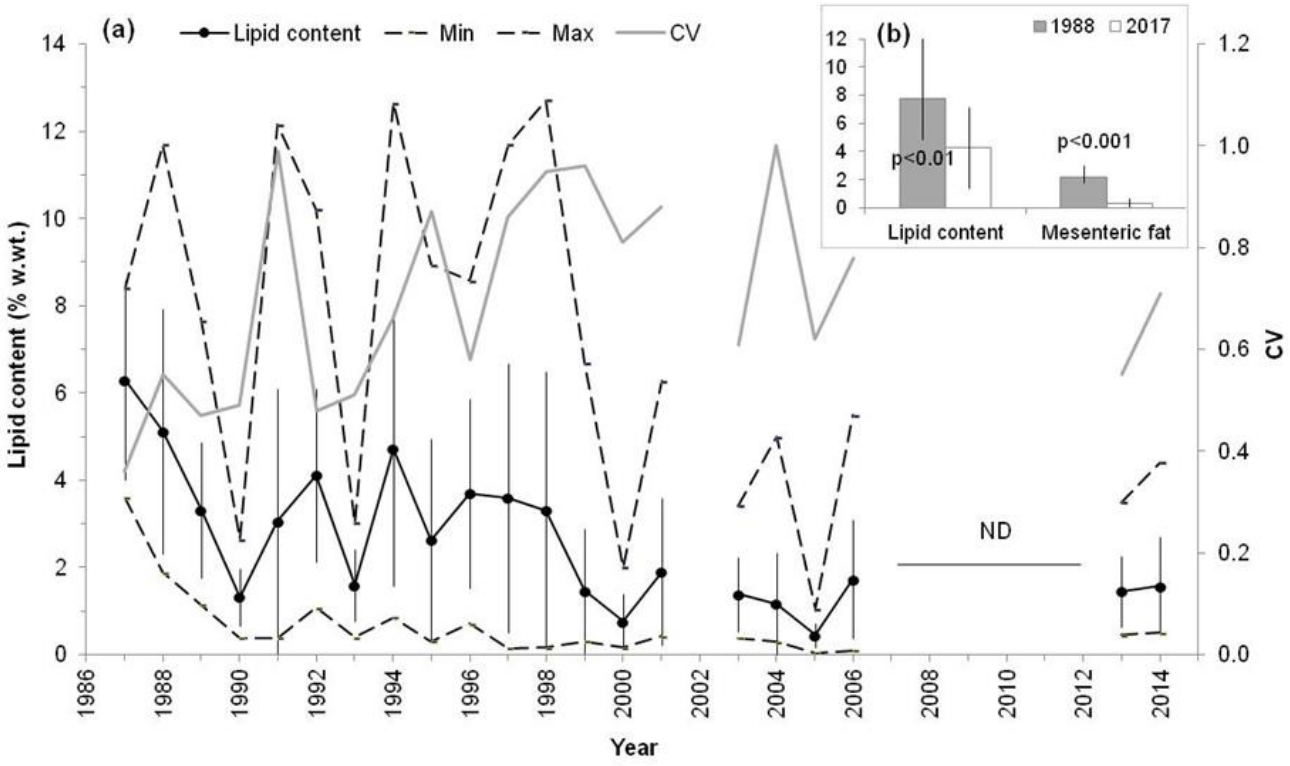

Figure 4 (a) Lipid content (% w.wt.) of the muscle tissue in spawning Baltic Herring females in the Archipelago Sea in 1987–2006 and 2013–14, and (b) lipid content (% w.wt.) and amount of mesenteric fat (relative scale from 0 to 4) in herring samples collected in winter from the Archipelago Sea in 1988 and 2017. In the large panel, bold solid line indicates the sample means with standard deviation (vertical bars), dashed lines the maximum and minimum values in the samples and grey line the coefficient of variation (CV). In the small panel, values of *p* indicate the significance level in comparisons between 1988 and 2017 (Mann-Whitney U test). ND = no samples were obtained.

Fig. 5

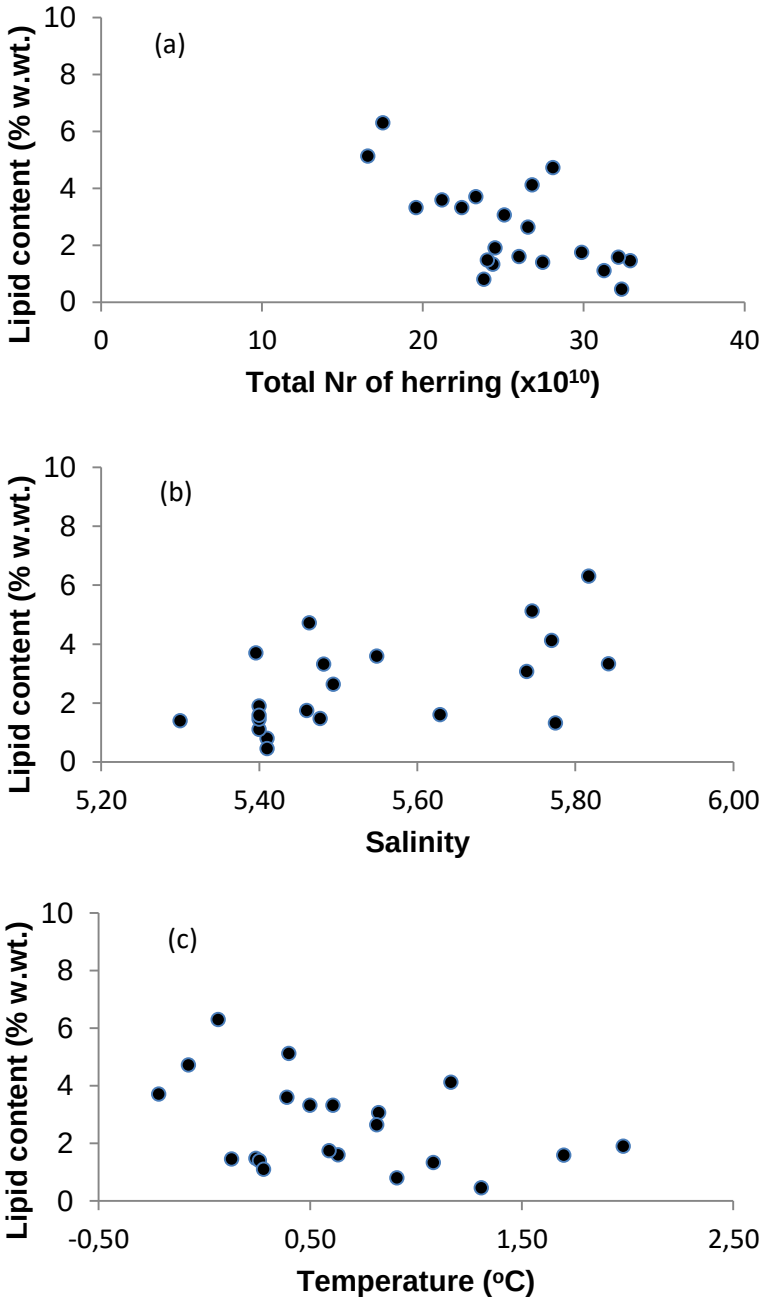

Figure 5 Relationship between the lipid content of the muscle tissue in spawning Baltic Herring females and (a) total number of herring in the Bothnian Sea; (b) mean SW salinity and (c) mean SW temperature in January-April; time series from 1987-2006 and 2013-14.

Table 1 Number (n) and mean total length (cm; standard deviation in parenthesis) of spawning and overwintering female Baltic Herring (*Clupea harengus membras*) examined for the lipid content of the muscle tissue during 1987-2017. - = no data. *) indicates the years when lipid content showed a significant and positive correlation with fish length in the samples (significance level $p<0.05$; Spearman rank correlation test).

Year	*n*	*Length (cm)*
Spawning		
1987	5	17.7 (1.9)
1988	20	19.8 (2.6)*
1989	20	19.0 (2.6)*
1990	15	18.8 (1.4)
1991	15	19.1 (2.0)
1992	29	17.6 (2.3)
1993	15	18.6 (2.4)
1994	20	21.0 (3.6)*
1995	20	18.8 (2.3)
1996	15	18.8 (2.3)*
1997	31	18.2 (2.4)
1998	19	17.2 (3.1)*
1999	22	18.2 (2.4)
2000	10	16.4 (2.7)
2001	20	16.5 (1.7)
2002	-	-
2003	15	16.6 (2.1)
2004	15	16.4 (1.6)
2005	12	16.2 (2.0)
2006	22	16.2 (1.6)
2007-2012	-	
2013	17	16.8 (1.4)
2014	14	16.5 (1.5)
Total	**371**	
Winter		
1988	28	17.6 (1.7)
2017	20	15.9 (1.4)
Total	**48**	

Table 2 Generalized Linear Mixed Model parameter estimates showing the effect of year (Model I) on the lipid content (% w.wt.) of the muscle tissue of the Baltic Herring (*Clupea harengus membras*), sampled in the Archipelago Sea during 1987–2006. Model (II) shows the effects of salinity (S), total number of Baltic Herring (TotNr), and temperature in January–April ($T_{Jan–Apr}$) on the lipid content (% w.wt.) during the same period. In bold are those values judged to be significant ($p<0.05$); n indicates the number of observations used by the models.

Solutions for fixed effects						
Effect	n	Estimate	SE	DF	T	p
Model I						
Year	340	-3.98	0.16	321	-25.62	**<0.001**
Model II						
	340					
Intercept		9.89	9.85	335	0.32	0.32
S		0.92	0.36	336	2.56	**0.01**
$T_{Jan-Apr}$		-0.24	0.11	336	-2.15	**0.03**
TotNr		-1.76	0.75	335	-2.34	**0.02**

Table 3 Relative proportions of major fatty acids (% of total FAs; mean±SD) in the muscle tissue of the Baltic Herring (present study) and in adult *Limnocalanus macrurus* (Mäkinen et al. 2017) in the study area in 2013. The samples were collected from the southern Bothnian Sea (BS) and the Archipelago Sea (AS) in 2013. n = number of samples analysed; ND = not detected; +) = proportion <0.5 % (included in the group "Others").

	Herring			***Limnocalanus*** [1)]	
Area	BS	AS	BS	BS	BS
Date	June 3	June 17	Sept 26	June 12	Sept 9
	(n=5)	*(n=5)*	*(n=5)*	*(n=1)*	*(n=3)*
Fatty acid	Mean±SD	Mean±SD	Mean±SD		Mean±SD
14:0	3.0±0.7	3.5±1.0	3.2±0.3	2.7	1.5 ± 0.6
16:0	21.5±1.0	21.7±0.7	21.9±1.1	9.0	8.7 ± 1.4
18:0	2.4±0.8	2.2±0.4	1.7±0.9	2.9	2.4 ± 0.6
20:0	ND	ND	ND	0.8	+
22:0	0.9±0.6	0.4±0.2	0.2±0.1	ND	ND
SAFA Total	*27.8±1.0*	*27.8±1.3*	*27.0±1.6*	*15.4*	*12.6±2.6*
16:1n-7	6.0±3.2	6.3±2.5	7.0±1.8	1.1	1.6 ± 0.1
18:1n-7	3.7±0.9	3.9±0.9	4.0±0.4	1.0	1.8 ± 1.4
18:1n-9	12.6±2.5	18.6±7.1	14.7±3.8	6.3	15.6 ± 2.1
22:1n-9	ND	ND	ND	0.8	0.8 ± 0.2
24:1n-9	1.1±0.2	1.4±0.5	1.1±0.1	1.3	1.0 ± 0.0
MUFA Total	*23.4±4.5*	*30.2±7.0*	*26.8±5.7*	*10.5*	*20.8±2.6*
18:2n-6	5.1±0.9	5.8±0.5	6.4±0.9	8.3	10.5 ± 0.8
20:2n-6	1.5±0.4	1.7±0.6	2.5±0.4	1.4	3.3 ± 0.8
20:4n-6	1.1±0.1	0.9±0.3	0.9±0.1	ND	ND
18:3n-3	2.2±0.9	2.0±0.6	2.7±0.4	4.3	4.6 ± 0.2
18:4n-3	2.3±1.5	1.5±1.0	2.3±0.6	3.9	3.5 ± 0.6
20:3n-3	0.7±0.4	0.7±0.3	1.6±0.4	1.0	2.8 ± 0.6
20:5n-3	9.0±0.7	7.0±1.8	7.9±1.0	22.9	10.1 ± 0.9
22:6n-3	21.1±6.9	17.5±5.4	14.9±3.4	9.9	16.1 ± 1.4
PUFA Total	*43.0±4.8*	*37.1±7.4*	*39.2±4.6*	*51.7*	*50.9±5.1*
Others	5.9±2.0	4.9±0.7	6.9±0.5	22.4	15.4 ± 0.3
TOTAL	100.0	100.0	100.0	100.0	100.0

1) Each sample consists of 30-32 adult individuals

Table 4 Results of SIMPER analysis showing the average dissimilarity between the lipid composition (% total FA) of *L. macrurus* (I), and herring muscle tissue (II) in the samples collected in June and September 2013 from the southern Bothnian Sea and the Archipelago Sea.

Dissimilarity (%)	Fatty acid	Contrib. %	Av. % abund.		Sim/SD	Cum. %
			I	II		
30.60	16:0	6.46	8.80	21.72	9.77	20.45
	"Others"	5.60	17.11	5.92	3.31	38.16
	18:1n-9	2.78	13.30	15.28	1.36	46.96
	20:5n-3	2.69	13.28	7.95	0.94	55.48
	22:6n-3	2.55	14.57	17.84	1.05	63.55
	16:1n-7	2.51	1.46	6.43	2.23	71.49
	18:2n-6	2.10	9.99	5.79	2.84	78.13
	18:1n-7	1.12	1.61	3.85	1.81	81.69
	18:3n-3	1.09	4.52	2.33	3.14	85.15
	18:4n-3	0.83	3.59	2.03	1.64	87.77
	14:0	0.75	1.81	3.25	1.75	90.15
	20:3n-3	0.72	2.33	1.00	1.57	92.42
	20:2n-6	0.62	2.83	1.89	1.53	94.37
	20:4n-6	0.47	0	0.93	4.46	95.85
	22:1n-9	0.39	0.77	0	4.85	97.07
	18:0	0.37	2.53	2.07	1.13	98.24
	22:0	0.24	0	0.48	1.10	99.00
	20:0	0.20	0.40	0	1.87	99.64
	24:1n-9	0.11	1.10	1.22	0.90	100
19.67	SAFA	7.98	13.5	27.67	6.19	40.58
	PUFA	6.56	51.25	39.80	1.74	73.94
	MUFA	4.07	18.25	26.80	1.25	100