Formation of lipid reserves, fatty acid composition and reproduction of *Limnocalanus macrurus* (Copepoda, Calanoida) during summer in the Bothnian Sea, northern Baltic Sea

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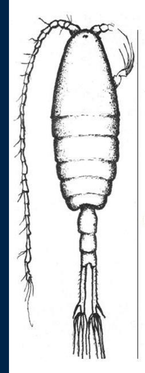
BACKGROUND



Limnocalanus macrurus is a cold-stenothermic copepod that in the Baltic Sea occurs abundantly in low-salinity areas, such as the Bothnian Bay and the Bothnian Sea, where it is one of the most important species of the pelagic ecosystem.

Projections for the future of the Baltic Sea suggest that a further decline of salinity will enable the species to expand its distribution southward to the Central Baltic, where it eventually could replace copepod species, e.g. *Pseudocalanus acuspes*, having higher salinity requirements. Lipids may get a central role in this process as they are crucial in the production and transfer of energy in the food webs.

We studied the formation of the lipid reserves in *L. macrurus* in the Bothnian Sea, with a special emphasis on the fatty acid composition in reproductive adults and their potential food. Our primary interest was the spring and summer season, as it is the main production and feeding period of the plankton community in the southern Bothnian Sea. The study was started at the time of the spring bloom of phytoplankton during May and it was continued until September, when the plankton production ceases.

OBJECTIVES

• Determine the lipid content of adult *L. macrurus* over summer (May, July, and September)

• Study the seasonal pattern of the fatty acid (FA) composition in an adult *L.macrurus*

• Compare the fatty acid composition between *L. macrurus* and a mixed plankton samples to investigate trophic relationships between *L. macrurus* and its food.

MATERIALS AND METHODS

Sampling and measurements

• *L.macrurus* samples were collected for lipid and fatty acid analyses at station 1 (Fig. 1) during May 23, July 1 and September 9, 2013

• A mixed-plankton sample was collected for fatty acid analyses in May 23, 2013

• In 2014, the vertical and horizontal occurrence of *L.macrurus* was studied by collecting samples of *L.macrurus* at 3–5 week's intervals at the deep (St. 1; 50 m) and shallow (St. 2; 25 m) study sites. From these samples, the body length, size of oil globules, and the presence of spermatophores in adult females was also recorded.

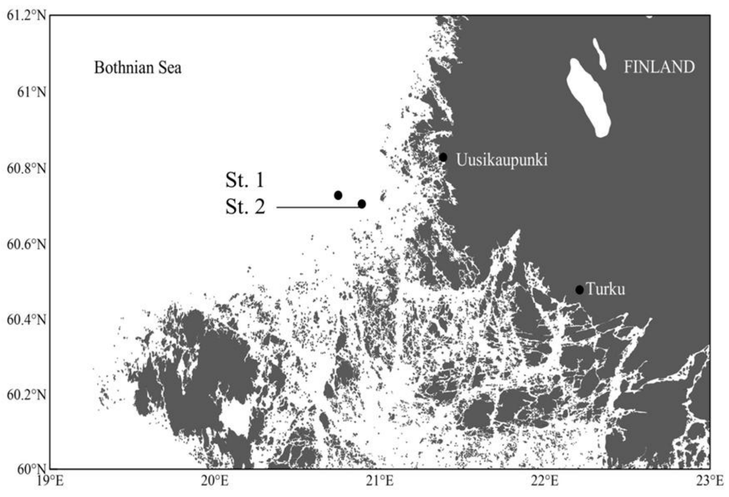


Fig. 1. Sampling points (St. 1 and St. 2) in the southern Bothnian Sea. (SW Finland).

Extraction of lipids and preparation of fatty acid methyl esters

Lipids were extracted from the samples of *L.macrurus* (n=30-32 ind./sample; 3 replicates/date) and mixed-plankton (pooled) by a modified Folch method using chloroform/methanol (2:1, v/v). Fatty acid methyl esters (FAME) were analysed with gas chromatography.

Statistical analyses

The data was analysed using ANOVA, Principal component analysis (PCA), and Analysis of Similarity (ANOSIM). The analyses were done with the vegan package in R statistical software.

PRELIMINARY RESULTS

Vertical distribution and reproduction

During 2014, adult *L.macrurus* were present only at station 1 (St.1; 50 m). In the shallower sampling station (St.2; 25 m), adults were missing and copepodites were present only during May. In general, nauplii were distributed evenly in the water column, while the older developmental stages were confined to deep water when water temperature at the sea surface started to rise.(Fig. 2).

Spermatophores were found in 0-5% of the females indicating that some reproduction took place during May–September. This was also seen as minor peaks in the abundance of nauplii during July and September 2014.

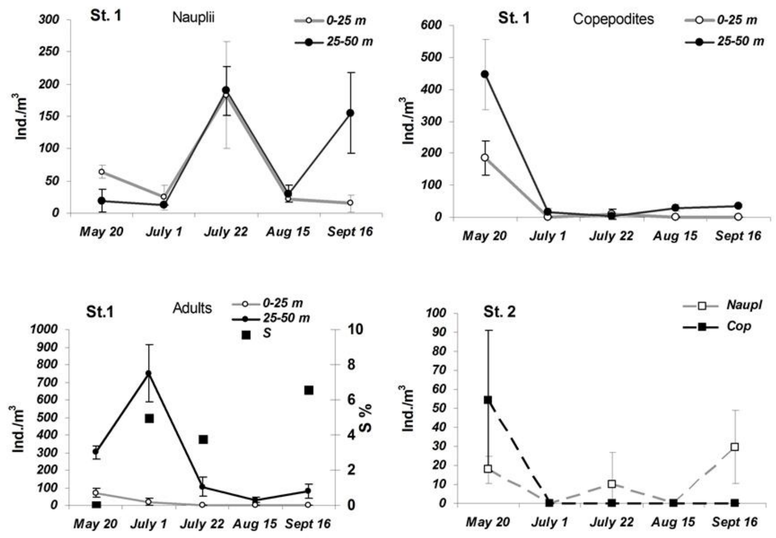


Fig. 2. Abundance (ind./m³; mean and ±SD) of *L. macrurus* nauplii, copepodites and adults at stations 1 and 2, and presence of spermatophores (S% of females examined) during May–September 2014. Note different scales of y-axis.

Lipid and fatty acid analyses

In 2013, the lipid content of an adult *L. macrurus* decreased from an average of 7.7 µg in May to 3.0 µg in July and 4.9 µg in September. The same decreasing trend was also detected in 2014 from the total size of oil globules. As this trend was not related to the body size, the results indicate that the high lipid reserves during May resulted from good feeding conditions provided by the phytoplankton spring bloom in surface waters.

PCA and ANOSIM (R=0.74, p=0.02) separated the FA samples by season, showing the largest separation between May and the two other months (Fig 3). In May, *L. macrurus* contained lower proportions of EPA (20:5(n-3)), DHA (22:6(n-3)), PUFAs, 20:0, and LIN (18:2n-6) than during July through September (Table 1). To verify these results, some of the the fatty acid analyses will be further analysed with an Gas-Chromatography–Mass Spectrometry (QC-MS) analysis.

The mixed-plankton sample included fewer FAs than *L. macrurus* and, for some FAs, differed from *L.macrurus* (Table 1). During July-September, *L. macrurus* were dominated by dinoflagellate markers LIN and SDA (18:4(n-3)) (Table 1; Fig. 4), which corresponded to the species composition of the analysed sample.

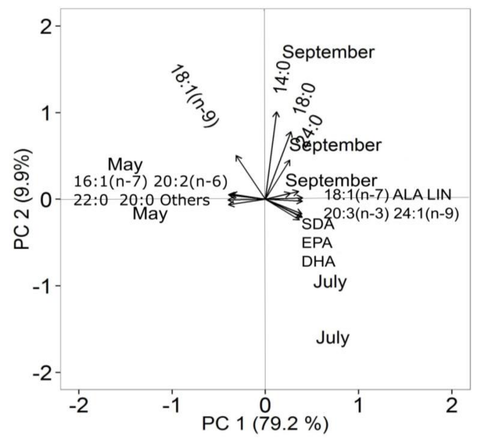


Fig 3. PCA Biplot of the fatty acid proportions (FA; weight percentage of total fatty acids) of *L.macrurus* during May, July and September 2013. Distance between fatty acids on the biplot represents Euclidean distance in multidimensional space.

Table 1. Relative proportions of fatty acids (FA; weigth percentage of total fatty acids) in *L. macrurus* and mixed-plankton in 2013 in the Bothnian Sea. *n* = number of samples per sampling date.

	L. macrurus						Mixed-plankton
	May 23 *n*=3		July 1 *n*=3		September 9 *n*=3		May 23 *(pooled)*
FA	Mean	± SD	Mean	± SD	Mean	± SD	Mean
14:0	0.2	0.4	0.2	0.2	1.1	1.0	11.6
16:0	3.8	0.2	11.1	1.5	8.9	1.4	17
18:0	10.5	0.5	11.6	1.4	15.9	2.2	0.7
20:0	9.6	0.4	0		0.3	0.7	4.1
22:0	6.7	0.7	2.7	1.0	3.2	1.5	0
24:0	0.2	0.0	0.7	1.0	2.0	0.6	0
∑SAFA	**31.0**	**2.2**	**26.2**	**5.1**	**31.5**	**1.2**	**33.4**
16:1(n-7)	6.0	0.5	1.5	0.4	1.6	0.1	22.9
18:1(n-7)	0.5	0.1	1.8	0.3	1.8	1.4	0
18:1(n-9)	2.0	0.4	0.0		0.5	0.7	8
22:1(n-9)	1.0	0.2	1.1	0.2	0.8	0.2	0.7
24:1(n-9)	0.3	0	0.9	0.0	1.0	0.3	0
∑MUFA	**9.8**	**1.2**	**5.2**	**0.9**	**5.8**	**2.7**	**31.6**
20:2(n-6)	7.9	0.7	2.2	0.8	3.4	0.8	0
20:3(n-3)	0.5	0.3	2.0	0.5	2.8	0.7	0
18:2(n-6)	2.0	0.4	11.0	1.4	10.7	0.9	1.7
18:3(n-3)	1.1	0.3	5.0	0.4	4.7	0.2	1.5
18:4(n-3)	1.2	0.3	4.3	0.2	3.6	0.6	0
20:5(n-3)	3.2	1.0	11.9	0.6	10.3	1.0	12.1
22:6(n-3)	4.4	1.3	19.1	1.6	16.5	1.4	8.5
∑PUFA	**20.4**	**4.3**	**55.5**	**5.5**	**51.9**	**5.6**	**23.8**
Others'	38.8	3.1	13.0	3.9	10.8	1.5	5.9

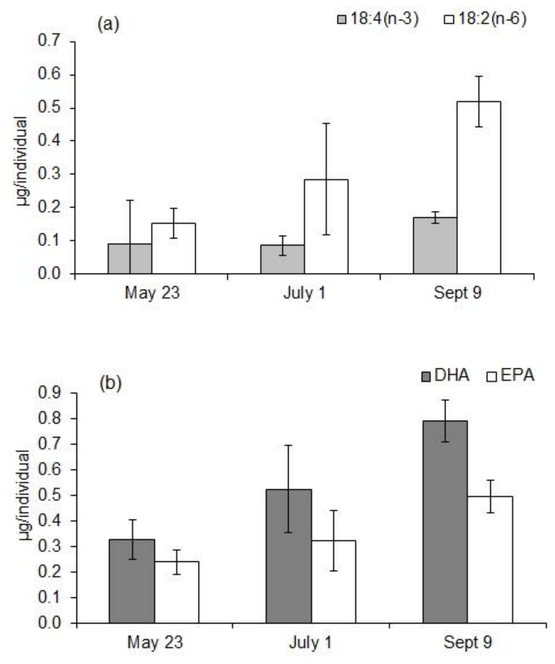


Fig 4. Fatty acid contents (µg/individual; mean±SD) of adult *L.macrurus* during May, July and September 2013. Panel (a) shows the fatty acid markers suggesting flagellate diet and panel (b) the fatty acids essential in reproduction. *n* = 3/date; each sample consists of 30–32 adult *L.macrurus.*

CONCLUSIONS

- The patterns of reproduction suggest that there could be two different strategies in the *L. macrurus* population of the study area: "capital-breeders" and "income-breeders" (Bonnet et al. 1998), possibly as a strategy to diminish intraspecific competition for the food resources.

- The results indicate that the amount of lipids does not necessarily limit the reproduction of *L.macrurus* alone, but some another factor, such as their quality. This will be verified with the QS-MS analysis and by continuing the study until winter.

- The expansion of *L.macrurus* to the Central Baltic would improve the feeding conditions of planktivorous fish like the Baltic herring (*Clupea harengus membras*), as, shown by our study, an adult *L. macrurus* contains significantly more lipids (2.9–7.7 µg/ind.) than *P.acuspes* (0.9–1.8 µg/ind.; Peters et al. 2006), the preferred prey of the herring in the Central Baltic during periods of high salinity (Möllmann et al. 2005). However, the rising sea water temperature may force *L. macrurus* to submerge permanently to deeper water where the concentration of food is low. If also the quality of food is poor, the growth of the population is most likely suppressed, which eventually limits the dispersal of the species to other regions in the Baltic Sea.

LITERATURE

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