GEOGRAPHIC DYNAMIC OF LIPID CONTENT LARVAE DAUBED SHANNY *LEPTOCLINUS MACULATUS* (FAMILY: STICHAEIDAE) FROM ISFJORDEN AND KONGSFJORDEN, SVALBARD

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Lipids for Arctic organisms very important as variant of adaptive behavior, pelagic larvae and fish feeding on *Calanus* ssp. diet in Arctic ecosystems and store a lot amounts of lipids from it during short summer productivity season. In general triacylglycerols are the usual depot of fat in pelagic fishes including all the commercially important species, whereas alkyl lipids including wax ester and alkyldiacylglycerols are widespread in meso- and especially bathypelagic fishes (Sargent et al., 1983; Falk-Petersen et al., 1986).

Daubed shanny *Leptoclinus maculatus* is among the dominating species of the epibenthic fauna of Arctic waters and thus play a significant role both as predators and prey in the Arctic ecosystem. The larvae are pelagic while the adults are bottom inhabitants. Pelagic larva is very common in pelagic biomass during whole summer season and they stay pelagic till 3 years old. Significant detail of *L. maculatus larva* is that it have ability to store lipids obtained from *Calanus* ssp. diet in particular part of the body called *lipid sac* situated on the ventral part of the body. Lipid sac as important body part have been investigated in several species of Antarctic fishes (DeVries, Eastman, 1977; Clarke et al., 1984; Friedrich, Hagen, 1994) and shown hot importance of it for adaptation, buoyancy and development in severe Antarctic conditions but no relevant works have been conducted about Arctic fishes. Storage, using and utilization of lipids in the larvae organisms appear high top interest as help to understanding of lipid dynamics in food web chain and food chain relationships, life cycle of larvae *L. maculatus* (turning from pelagic larva to bottom adult) because this sides of study have not been described yet.

The objective of this study is investigation of lipid classes and fatty acid spectrum qualitatively and quantitatively of larvae *L. maculatus* from Svalbard waters along the Northern-West coast of Spitsbergen from summer season.

Matherial and methods

The samples of larva *L. maculatus* was collected in Isfjorden(78^{0}20'N 15^{0}00'E), Kongsfjorden (79^{0}00'N 11^{0}40'E) and outside of Kongsfjorden – V10 (78^{0}95'N 08^{0}81'E) station during summer 2007 (22-29 July) at stations with depth 50 m with pelagic trawling on board RV "Jan Mayen". Weighed samples (0.5-1.0 g) of lipid sac and flesh from same in length and weight parameters from fresh larvae were derived from fresh material as soon as possible after capture of the fish and homogenized (where conditions permitted) in 10 volumes (10 ml) of 96% ethyl alcohol mixed with 0.001% of the antioxidant ionol. Sample homogenates were placed in plastic vials and stored onboard in a cooling room at 4 °C until delivery to the laboratory. The material was then fixed in a solvent system of chloroform: methanol (2:1, v/v), and total lipids (TL) were extracted following the method of Folch et al. (1957).

<u>Lipid class and fatty acid analysis</u>

The material was then fixed in a solvent system of chloroform: methanol (2:1, v/v), and total lipids (TL) were extracted following the method of Folch et al. (1957). Individual lipid classes were identified as phospholipids (PL), triacylglycerols (TAG), cholesterol (CH), and wax esters (WE) by thin-layer chromatography. Their quantities were determined using the hydroxamate method (Sidorov et al., 1972b) and then spectrophotometry. Quantitative determination of CH was determined based on Engelbrecht et al. (1974) and spectrophotometry.

The fatty acid and alcohol compositions of the total lipid extracts and the lipid classes were analysed by gas-liquid chromatography.

For all lipid data One-way ANOVA was performed. Due to the robustness of the ANOVS we assume that all data are normal distributed with even homogeneity of variance.

Results

Larva *L. maculatus* is a very lipid-rich fish the main functions of it accumulation of storage and metabolic lipids and in addition to it obvious role in buoyancy. The composition of the total lipids from the oil sac and the flesh is presented in Table 1. The maximum lipid content was determined in larva lipid sac from Kongsfjorden – 64,43% dry weight and minimum content in lipids sac from Isfjorden. The maximum lipid concentration noticed in larva muscle from Kongsfjorden and minimum outside of Kongsfjorden, deep station V10 – 27,95% and 24,48% dry weight. Triacylglicerols were dominant lipid compound in lipid sac and muscle: from 40,55 till 58,31% dry weight (from 93,36 till 89,72% sum lipids) and from 12,66 till 14,54% dry weight (from 54,62 till 45,33% sum lipids), respectively. The wax esters have not been occurred in lipid sac while in muscle composed a little content compare to triacylglicerols (Table 1). The same results were shown in Falk-Petersen with colleagues investigation (Falk-Petersen et al., 1986). Among polar lipids cholesterol (Chl) was dominant lipid component in muscle and totally absent as wax esters in lipid sac. The molar ration of Chl to phospholipids (PL) in muscle varied from 0,47 to 0,90 along the series: V10 station to Kongsfjorden via Isfjorden (Table 1). A high ratio in larvae muscle from V10 station out of Kondsfjorden was due to the fact that cholesterol regulates the fluidity of membrane and activation of membrane-connected enzymes. The main PL found in muscle and lipid sac were phosphotidylcholine (PC) and phosphotidylethanolamyne (PE), always they are dominant individual phospholipids in all studied organs and tissues of the fish (Velansky & Kostetsky, 2007). The ratio PC to PE reflects compensatory mechanisms that allow maintenance of physical-chemical membrane properties with changing temperatures (Kattner et al., 2007). Other PL found in muscle and lipid sac were in minor concentration (Table 1).

Table 1

Lipid class composition in muscle and lipid sac *Leptoclinus maculatus* larvae from Isfjorden, Kondsfjorden, V10 station from summer season (July, 2007)

Season	Summer					
Place	Isfjorden	Kongsfjorden	V10 St	Isfjorden	Kongsfjorden	V10 St
	Muscle			Lipid sac		
DWt	73,76±5,54	72,05±3,50	75,52±5,32	56,57±5,54	35,57±3,50	49,50±5,32
TL, % dwt	26,24±5,54	27,95±3,00	24,48±2,84	43,43±2,45	64,43±2,14	50,50±9,01
% dry weight						
Neutral lipids						
TAG	13,97±2,86	12,66±1,83	14,54±4,44	40,55±2,32	58,31±3,07	46,41±7,71
WE	1,89±0,32	1,74±0,36	1,89±1,51	0	0	0
Polar lipids						
CH	7,31±1,96	3,96±1,15	1,43±0,55	0	0	0
PL	3,06±0,78	9,58±1,35	6,62±1,16	2,88±0,17	6,08±1,67	4,09±2,19
% total phospholipids						
PI	0,50±0,03	2,34±1,02	2,17±0,19	0,18±0,00	1,09±0,14	1,10±0,37
PS	0,87±0,13	1,00±0,11	1,11±0,07	1,14±0,04	1,99±0,12	1,43±0,17
PEA	15,42±1,73	20,02±1,77	19,91±0,62	16,53±0,18	18,13±1,25	9,98±1,67
PC	81,87±1,92	74,47±2,64	75,83±0,52	81,24±0,25	75,22±2,14	79,80±2,80
LPC	0,22±0,03	0,27±0,07	0,42±0,02	0,44±0,05	0,77±0,17	5,55±1,78
SFM	0,33±0,01	0,38±0,03	0,32±0,03	0,05±0,01	0,49±0,08	2,15±0,96
CH/PL	0,50	0,47	0,90	0	0	0
% sum lipids						
Neutral lipids						
TAG	54,05±1,81	45,33±2,62	54,62±5,88	93,36±0,23	89,72±3,22	93,07±2,70
WE	9,27±3,05	6,57±1,06	4,46±2,62	0	0	0
Polar lipids						
CH	26,32±3,26	11,55±2,19	5,29±1,07	0	0	0
PL	10,35±2,07	36,54±3,18	35,62±7,42	6,64±0,22	10,23±3,21	6,92±2,75

The fatty acid composition of the lipid classes in the lipid sac and the flesh is presented in Table 2. The lipid sac was dominated by monounsaturated fatty acids – 20:1 (n-9), 22:1 (n-9) and smaller 16:1 (n-7), 18:1 (n-9). The 20:1 (n-9), 22:1 (n-9) fatty acids were found in very large amounts in calanoid copepods (Lee et al., 2006; Kattner et al., 2007) and here derived from zooplankton diet which is the most important for larva in Svalbard waters. The 16:1 (n-7), 18:1 (n-9) fatty acids derived from diatom and dinoflagellate phytoplanktonic diet which could compose food for larvae in different seasons especially early spring during phytoplanktonic bloom. The major saturated fatty acid were 14:0 and 16:0 (Table 2). The flesh was also dominated by 20:1 (n-9), 22:1 (n-9), high concentration of 16:0 saturated fatty acid was noticed in flesh too. Considerably high amounts of 20:5 (n-3), 18:4 (n-3), 22:6 (n-3) were found. Most of saturated fatty acids are the main components of membrane and membrane lipid components as PL.

Table 2

Fatty acid (FA) composition (% FA) in muscle and lipid sac *Leptoclinus maculatus* larvae from Isfjorden, Kondsfjorden, V10 station from summer season (July, 2007) (% FA)

Season	Summer					
FA/Place	Isfjorden	Kongsfjorden	V10	Isfjorden	Kongsfjorden	V10
	Muscle			Lipid sac		
14:00	3,65±0,19	6,46±1,03	5,57±0,50	5,41±0,00	5,50±0,18	7,00±1,10
16:00	13,39±0,99	12,48±0,46	12,09±0,76	5,56±0,11	7,92±0,16	9,81±1,95
18:00	2,05±0,14	2,06±0,07	1,98±0,01	0,99±0,09	1,25±0,04	1,45±0,16
20:00	0,97±0,14	0,26±0,06	0,31±0,18	3,98±0,00	4,05±0,90	0,38±0,00
24:00	0,58±0,01	0,30±0,05	1,57±1,40	0,47±0,05	0,49±0,06	0,43±0,06
Sum SAFA	20,65±1,16	21,56±1,04	21,52±0,84	16,41±2,02	19,22±1,01	19,08±3,27
14:1(n-5)	0,55±0,05	0,32±0,04	0,21±0,11	0,47±0,03	0,46±0,01	0,44±0,07
14:2(n-7)	0	0,04±0,02	0	0,16±0,02	0,12±0,03	0,17±0,03
15:1(n-5)	0	0,12±0,04	0	0,24±0,03	0,26±0,03	0,11±0,04
16:1(n-9)	0	0,004±0,00	0	0	0	0
16:1(n-7)	5,86±0,50	6,21±0,52	6,05±0,28	8,11±0,22	8,41±0,31	6,65±0,26
18:1(n-9)	6,80±0,48	6,18±0,24	6,01±0,34	5,03±0,28	4,54±0,12	5,43±1,01
18:1(n-7)	2,34±0,27	2,12±0,10	1,71±0,04	1,41±0,07	1,43±0,60	0,90±0,08
18:1(n-5)	0,88±0,07	0,70±0,06	0,46±0,23	0,56±0,08	0,49±0,08	0,70±0,05
20:1(n-9)	19,55±2,69	18,32±1,12	19,93±1,17	27,89±1,11	23,73±0,42	20,27±2,31
20:1(n-7)	1,21±0,34	0,58±0,07	0,63±0,23	1,01±0,07	0,58±0,04	0,53±0,09
22:1(n-11)	15,48±2,61	13,93±0,89	10,27±2,01	19,17±1,14	19,80±0,51	16,59±2,95
22:1(n-9)	3,64±0,23	1,65±0,09	1,85±0,33	1,78±0,05	1,68±0,24	1,35±0,03
24:1	1,11±0,10	0,61±0,07	0,36±0,15	2,34±0,24	2,01±0,52	3,23±0,37
Sum UFA	57,99±6,35	51,30±1,90	47,80±0,72	68,58±3,98	63,92±0,84	56,91±4,69
16:2(n-9)	0,35±0,10	0,27±0,08	0,50±0,30	0,11±0,00	0,23±0,10	0,06±0,04
Sum (n-9)	0,35±0,10	0,27±0,08	0,50±0,30	0,11±0,00	0,23±0,10	0,06±0,04
14:2(n-5)	0	0,05±0,03	0	0	0	0
16:2(n-6)	0	0,08±0,03	0	0,17±0,03	0,12±0,01	0,24±0,08
16:3(n-6)	0	0,42±0,23	0,37±0,34	0,19±0,03	0,18±0,03	0,21±0,03
18:2(n-6)	1,50±0,19	1,47±0,08	1,75±0,07	1,70±0,03	1,85±0,06	1,93±0,02
18:3(n-6)	1,14±0,36	0,13±0,04	0,14±0,11	0,32±0,09	0,14±0,01	0,40±0,14
20:2(n-6)	0,22±0,05	0,15±0,03	0,19±0,12	0,14±0,07	0,11±0,05	0,21±0,09
20:3(n-6)	0,27±0,06	0,29±0,09	0,31±0,22	0,31±0,04	0,29±0,06	0,17±0,03
20:4(n-6)	0,29±0,10	0,42±0,18	2,72±2,22	0,17±0,01	0,18±0,02	0,17±0,01
22:3(n-6)	2,02±0,67	0,24±0,15	0,66±0,45	0	0	0
Sum (n-6)	5,44±0,95	3,25±0,41	6,13±2,45	3,00±0,33	2,87±0,13	3,33±0,40
16:3(n-3)	0,22±0,07	0,23±0,04	0,09±0,05	0,23±0,06	0,14±0,03	0,43±0,07
16:4(n-3)	0	0,07±0,02	0,04±0,02	0,11±0,08	0,09±0,02	0,26±0,09
18:3(n-3)	0,97±0,00	0,87±0,07	1,09±0,13	0,89±0,07	0,96±0,10	1,15±0,05
18:4(n-3)	3,25±0,91	4,61±0,34	4,43±2,22	4,51±0,75	2,71±0,86	6,41±0,78
20:3(n-3)	0,27±0,27	0,13±0,04	0	0,17±0,04	0,04±0,02	0,18±0,06
20:4(n-3)	0,83±0,18	1,67±0,30	0,68±0,14	0,77±0,07	0,58±0,06	0,93±0,08
20:5(n-3)	4,43±2,09	6,62±1,09	7,88±0,29	4,07±0,44	4,64±0,24	4,01±0,66
22:4(n-3)	0,06±0,06	0,32±0,16	0,21±0,09	0,08±0,58	0,05±0,04	1,99±0,69
22:5(n-3)	0,32±0,08	0,56±0,09	3,94±3,48	0,45±0,04	0,65±0,35	0,34±0,01
22:6(n-3)	5,21±2,88	8,26±1,00	5,68±2,82	3,87±1,01	3,94±0,35	4,91±1,71
Sum (n-3)	15,58±6,05	23,33±2,15	24,04±1,51	15,15±1,00	13,80±1,30	20,60±1,06
Sum PUFA	21,36±5,19	27,14±1,97	30,68±0,83	18,26±1,01	16,85±1,38	24,00±1,42
Total FA	100	100	100	100	100	100
22:1/20:1	0,91±0,03	0,85±0,00	0,60±0,02	0,72±0,00	0,88±0,01	0,86±0,02

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LIFE HISTORY ASPECTS OF *LEPTOCLINUS MACULATUS* (STICHAEIDAE: LUMPENINAE) IN NORWEGIAN ARCTIC WATERS

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The species daubed shanny (*Leptoclinus maculatus*) is among the most abundant fish species in the epibenthic ichthyofauna of the Arctic waters of Norway, particularly in Svalbard fjords. Both larvae and adults have been found in relative high abundances along the ice edges inside fjords as well as the ice edge of North-East Svalbard, and thrive at temperatures below 0ºC. This species is likely an important component of Arctic food chain leading up to seabirds, but the life history of this species is still largely unknown. This study has resolved several life history aspects, including sex, size and age distribution, growth, age at maturity and reproduction. Samples of *L. maculates* were obtained from: Kongsfjorden, Isfjorden and its side-branches, Smeerenburgfjorden, Hinlopenstredet and Storfjorden. We also sampled specimens from the north-east ice edge of Svalbard, the southern Spitsbergen and the Barents Sea south to Bjørnøya. The sampling extended from April to October, and the material was pooled since there were no significant differences among areas or month of sampling except for reproduction. *Leptoclinus maculatus* is sexually dimorphic in size, growth and age of maturity. The populations are generally dominated by males. Length-weight relationship revealed that the males grow to a larger size than females (124 mm versus 113 mm), and both sexes have a positive allometric growth. There was no differences in age distribution between the sexes, but size-at-age analysis (von Bertalanffy growth function) showed that males are larger than the females at the same age and achieve a longer asymptotic length than the females. Age at maturity is about 4 years for males and 6 years for females. The gonadosomatic index of females increased from <5% in May to 20% in October. Histological investigations have shown that vitellogenesis and maturation phase occur in female ovaries at the end of October. Thus, spawning seems to take place in early winter, likely from November to January. Like most benthic fishes, *L. maculatus* has low fecundity and possesses large eggs. Differences in size-at-age and growth are likely to be caused by differential investment into reproduction, with females producing large, lipid rich eggs. During oogenesis, the quality and quantity of lipids in female gonads changed seasonally where increasing of phospholipids and triacylglycerols in ovaries (main classes of structural and energetic lipids) were important for growth and development of embryos. Both ovaries and liver were rich in lipids and raised from late winter to autumn from 32.7±2.1% to 78.5±4.3% and from 32.7±5.2% to 57.5±5.2% in the respective tissues. Triacylglycerols were the dominant neutral lipid during all seasons. The larvae migrate to the upper water layers, where they live pelagically for two years feeding on zooplankton in the summer and utilizing lipids stored in a large lipid sac during winter. When the lipid sac is depleted, the juveniles descend to the bottom and start a benthic life.