Institute of Biology of the Karelian Research Centre
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Ecological biochemistry lab

Biochemical studies in research of ecology of pink salmon:
lipids and fatty acids in biochemical adaptations

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Pink salmon meeting - 2018

Project: *Salmonids of the northwest of Russia: ecological and biochemical mechanisms of early development (2014-2016)*

The parameters of biochemical metabolism (lipid, protein, carbohydrate and energetic) in development of **the Atlantic salmon** (*Salmo salar* L.), **brown trout** (*Salmo trutta* L.), **pink salmon** (*Oncorhynchus gorbuscha* (Walb.)) reproducing and inhabited different biotopes of freshwater reservours in the northern-western part of Russia are studied including **all stages of the growth and development – from fertilization and embryogenesis to young's development in nature**

The project is important continuation on a physiological and biochemical level of population and genetic studies about the mechanisms of formation of genetic diversity and divergence during development, and an important continuation of

age and sub-population structure of Salmonid fish species

Pink salmon

Pink salmon (*Oncorhynchus gorbuscha* Walbaum, 1792) – a fish species from the Salmonidae family successfully introduced in freshwater rivers and streams of th Kola Peninsula

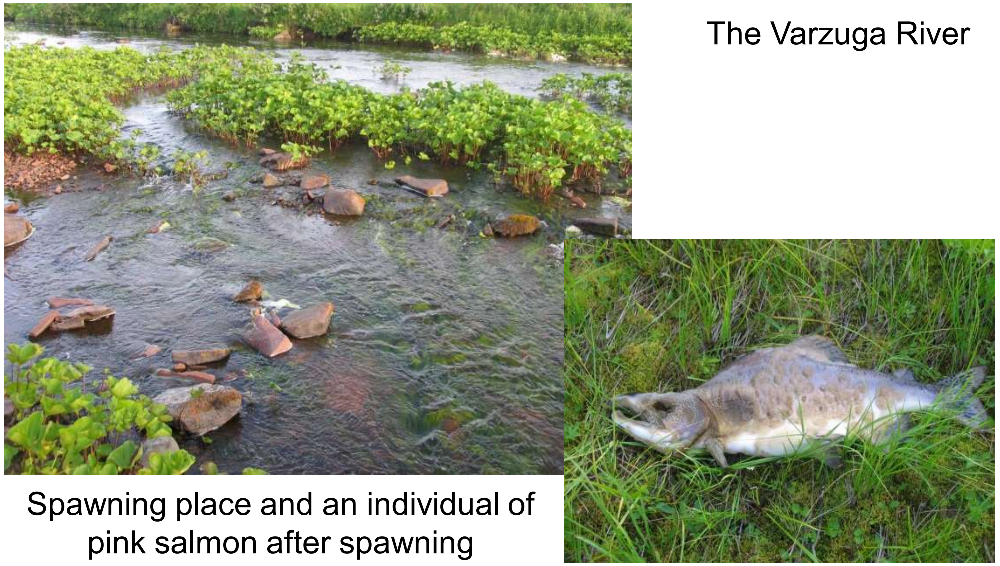
Spawning individuals

The Indera River

The Varzuga River

Самка горбуши.

Female and male of pink salmon in the Indera River



Spawning place and an individual of pink salmon after spawning

Lipids and other biochemical compounds that accumulate in the oocytes during oogenesis are used later by the developing embryos as structural and energetic elements. Salmonids eggs are rich by lipids. Lipids perform essential functions at all stages of ontogenesis with their functions largely determined by the fatty acid constituents

Among the main biochemical criteria for egg maturation and readiness for fertilization are the lipid content and the fatty acid composition of the egg. The levels and the ratios of certain lipid fractions and fatty acids are indices of the viability of offspring

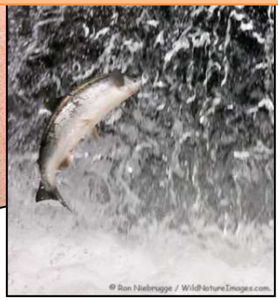
Habitation conditions (temperature, currencies, ground structure, food resources etc.) largely **affect the physiological and biochemical state** of aquatic organisms, including the level of lipids that have important functions in cellular metabolism.

WATER TEMPERATURE

FOOD

Knowledge of ecological and biochemical mechanisms of early development of salmonids is necessary to identify:

- the features of species development and adaptations,
- for determining the role of factors of biotope environment in FORMATION OF POPULATION STRUCTURE of the species IN EARLY DEVELOPMENT,
- the beginning of smoltification.



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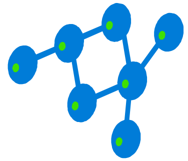
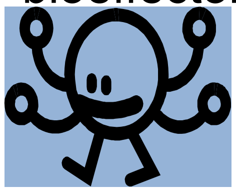
Classes and functions of lipids:



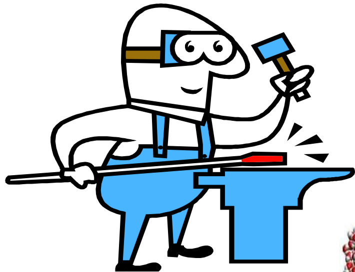
- The functional condition of both individuals and the entire population can be very accurately characterized using lipid parameters. The biochemical parameters of lipid metabolism are the most sensitive and mobile in terms of initial adaptive reactions that take place on the level of macromoleculs and cells as an answer to specific conditions of habitation in the northern latitudes;
- Individual lipid classes are multifunctional, and each function has a decisive role in specific environmental situations and the physiological condition of the organism

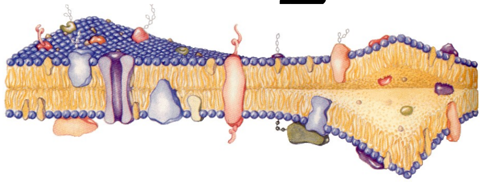
Energetic lipids
(Storage lipids)

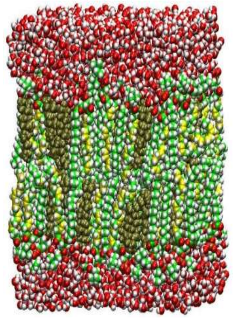
Lipids as
bioeffectors



Structural
components of
biomembranes







FATTY ACIDS (FA)

Fatty acids are the most sensitive lipid components that actively participate in the development of the compensatory reactions of organisms under normal conditions and under stress.

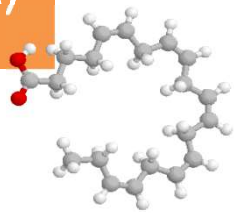
Rapid changes in the lipid and fatty acid profiles of fish tissues and organs contribute to optimizing metabolic processes and adapting to a changing environment. Lipid composition is a biochemical indicator of the status and health of embryos and juveniles and reflects processes such as the intra-population differentiation of fishes.

SATURATED FA (SFA)

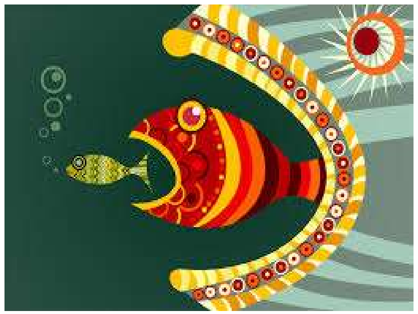
MONOUNSATURATED FA (MUFA)

POLYUNSATURATED FA (PUFA)

(n-3) (n-6) (n-9) (n-7)
families



It is known that the significant level of FA constituents of fish lipids derived from food. Thus, FA spectrum can reflect the specific food objects of the fish.
TROPHIC BIOMARKERS THEORY.



It is known that the significant level of FA constituents of fish lipids **derived from food**. **FA spectrum** can reflect the specific food objects and trophic interactions of the fish.

TROPHIC BIOMARKERS THEORY.

Energetic lipids
(Storage lipids)

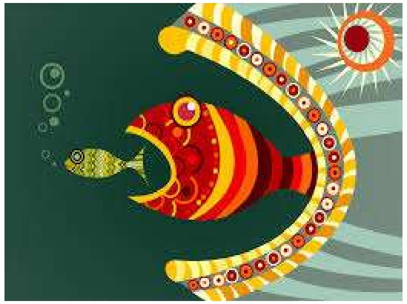
TAG and Wax

This approach has been used to investigate the trophic relationship from phytoplankton to fish in different geographical areas

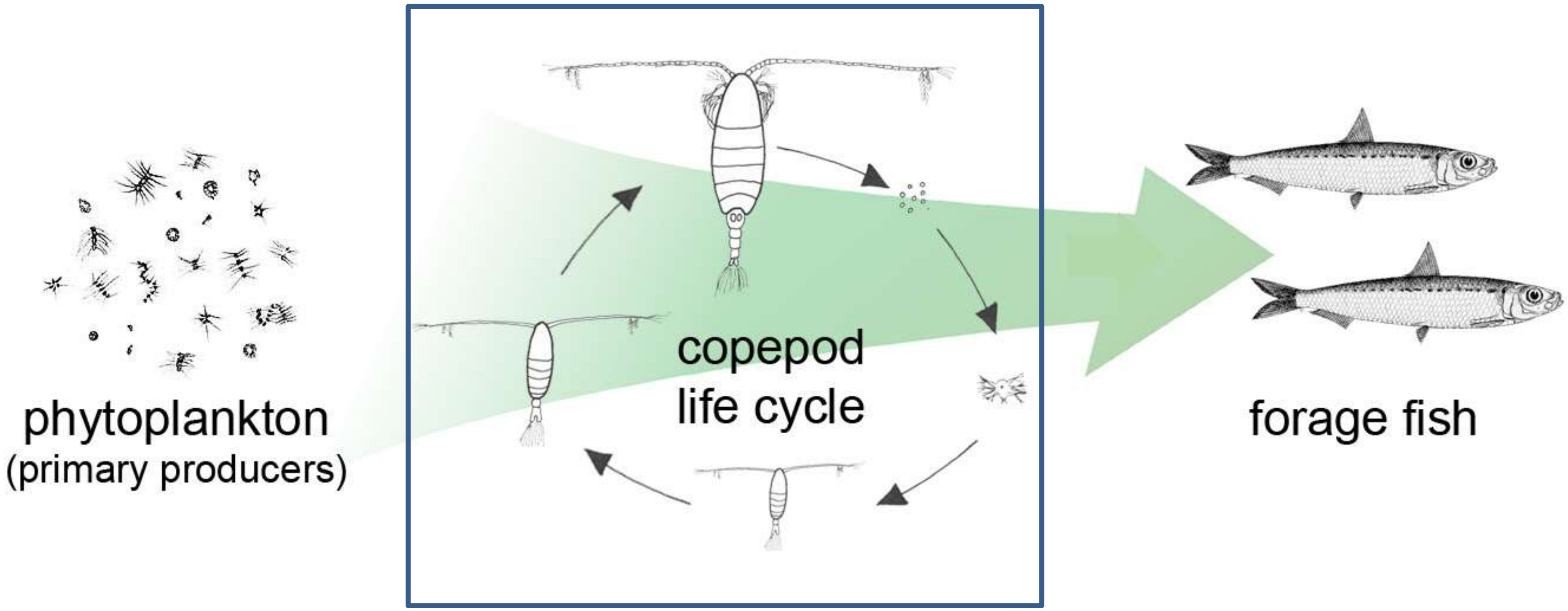
Fatty acid biomarker	Producer
16:1(n-7), 18:4(n-3), 18:1(n-7)	Phytoplancton
16:1(n-7), 22:5(n-3) and 20:5(n-3) – high amount 16:0/16:1(n-7)≥1	Diatoms (Bacillariophyceae)
18:1(n-9) 18:4(n-3) **18:5(n-3)** **22:6(n-3)**	Flagellates
20:1(n-9) and 22:1(n-11)	ONLY *Calanus* spp.
Prevalence of branched FAs and odd chain FAs	Bacteria
18:1(n-9)/18:1(n-7)	Carnivorous vs. herbivorous
18:1 and MUFA/PUFA	Aquatic organisms (fish) in the Arctic vs. temporal latitudes
16:0/18:1(n-9)	Metabolic rate

Fatty acids have been used in marine biogeochemistry as food chain biomarkers

The reserve lipids of **the primary consumers** are to a great extent **left unmodified** during trophic transfer, which makes them **useful** in identifying food sources and feeding models **on the short-term**



"Trophic biomarkers" in marine ecosystems of the high latitutdes



Diatoms	Copepods	Fish
16:1ω-7 **20:5ω-3**	**20:1ω-9** **22:1ω-11**	Branched FAs and odds FAs – bacterial food
Dinoflagelates		18:1ω-9/18:1ω-7 omnivorous vs. herbivorous
18:1n-9 18:4n-3 **22:6ω-3**		18:1 and MUFA/PUFA high latitudes fish *vs* Temper latitudes fish

Material and methods:
II. LAB work

- **STRUCTURAL LIPIDS**
  - •PHOSPHOLIPIDS
  - •CHOLESTEROL
- **ENERGETIC LIPIDS**
  - •TRIACYLGRLYCEROLS
  - •CHOLESTEROL ESTERS
- **FATTY ACIDS**

Phosphatidylinositol (PI)
Phosphatidylserin (PS)
Phosphatidylethanolamine (PEA)
Phosphatidylcholine (PC)
Lysophosphatidylcholine (LysoPC)
Sphingomyelin (SFM)

HPTLC
HPLC
GC

I. Biochemical heterogeneity on lipid status of female gonads of pre-spawning ping salmon *Oncorhynchus gorbuscha* (Walbaum 1792) (Varzuga River, White Sea Basin)

Pink salmon sampling in the Indera River

Sampling

Portions sampling.

Eggs of pink salmon

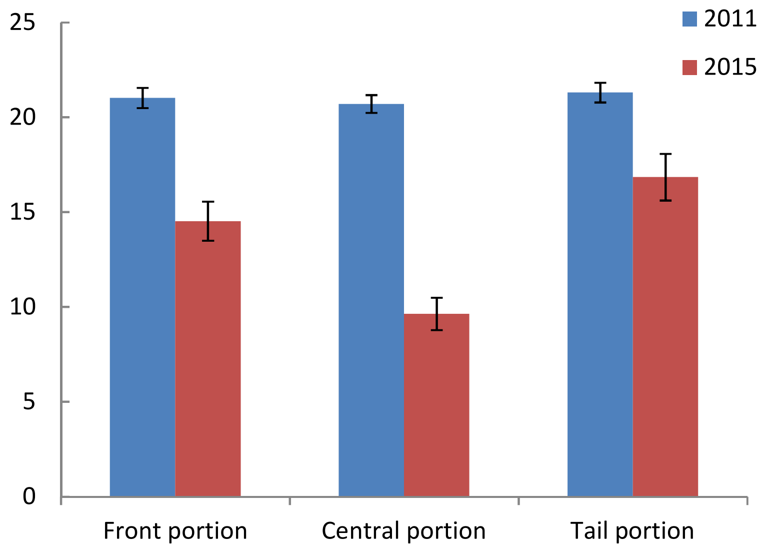
Самка горбуши в сетке.

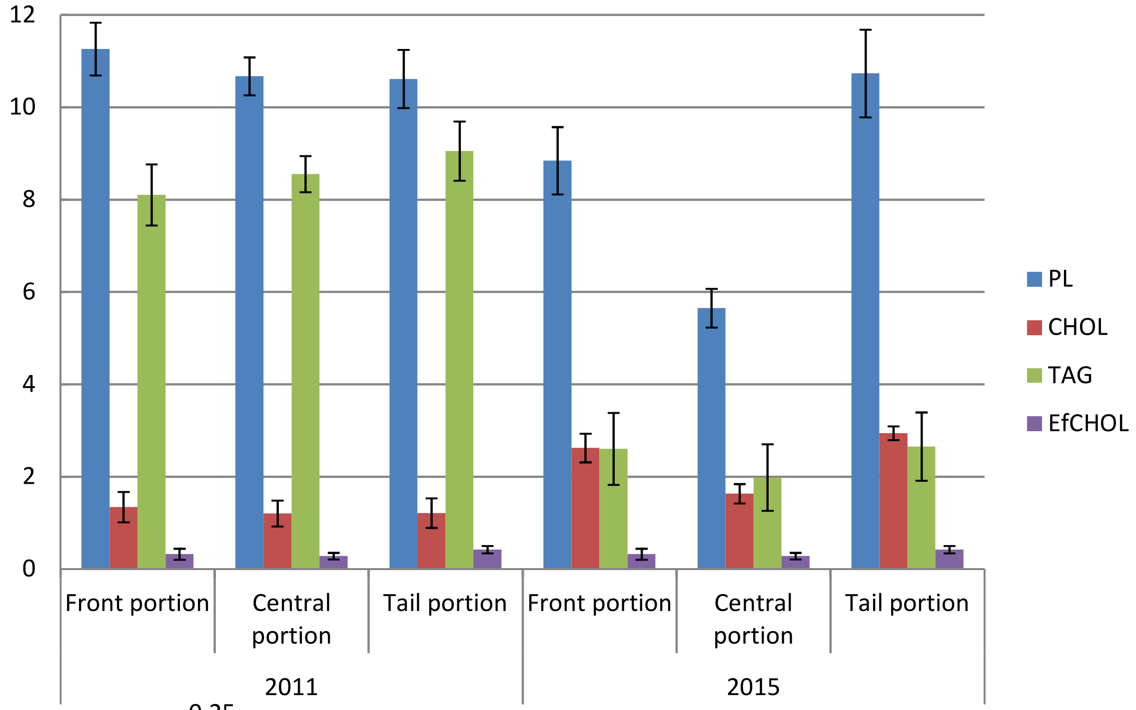
Самка горбуши.

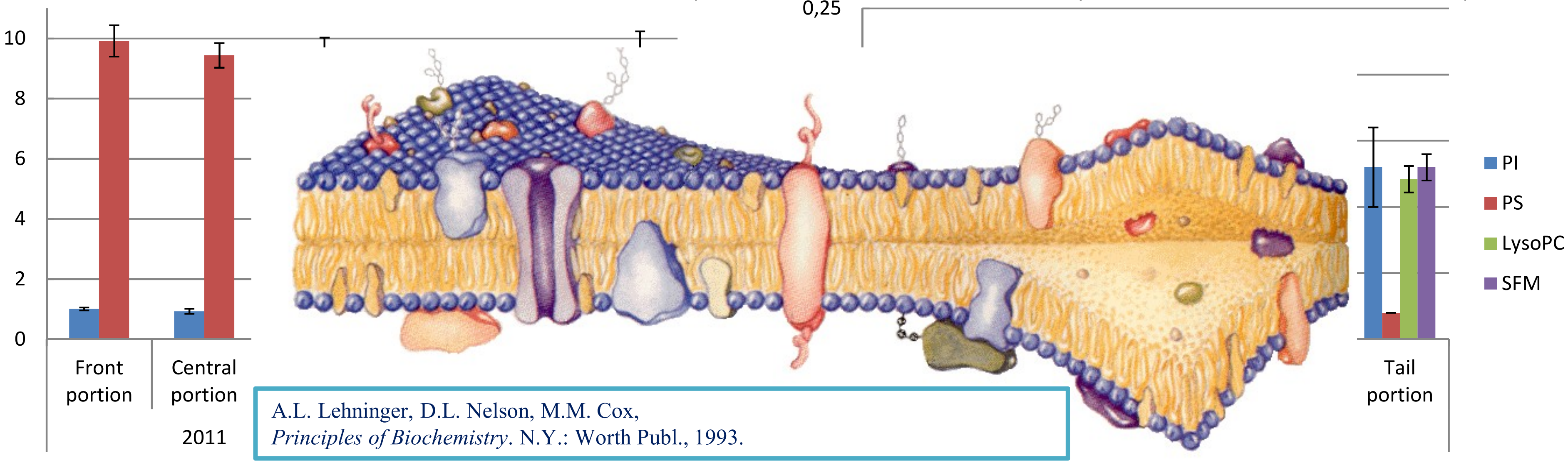
Comparative analysis of lipid and fatty acids contents in certain portions of female gonads – front, central and tail – of prespawning pink salmon *Oncorhynchus gorbusha* was made.

Total lipids (TLs) amount and lipid spectrum in front, central and tail portions of ovaries of the prespawning pink salmon in 2011 and 2015.

Total lipids amount – up to 21% dw.







A.L. Lehninger, D.L. Nelson, M.M. Cox,
Principles of Biochemistry. N.Y.: Worth Publ., 1993.

Fatty acids (% sum) profile in certain parts of pre-spawning ovaries

2011	Portions		
	Front	Central	Tail
18:1(n-9)	19,16±0,81^{B}	21,84±1,15^{A}	22,54±1,23^{A}
20:4(n-6)	0,97±0,06^{A}	0,95±0,03^{A}	0,87±0,07^{A}
(n-6			

It was determined the heterogeneity of eggs on lipid status of certain portions of ovaries: in front portion – high level of physiologically important eicosapentaenoic 20:5(n-3) and docosahexaenoic 22:6(n-3) fatty acids, which coincided with higher intensity of lipid metabolism on which higher ratio of 16:0/18:1(n-9) showed.

For example, it is known, that a tight correlation between the level of 22:6(n-3), docosahexaenoic fatty acid, in the lipids of adult gonads and the survival rates of the developing embryos post-fertilization and the larvae of fish has been reported (Kaitaranra and Linko 1984; Tocher 2003).

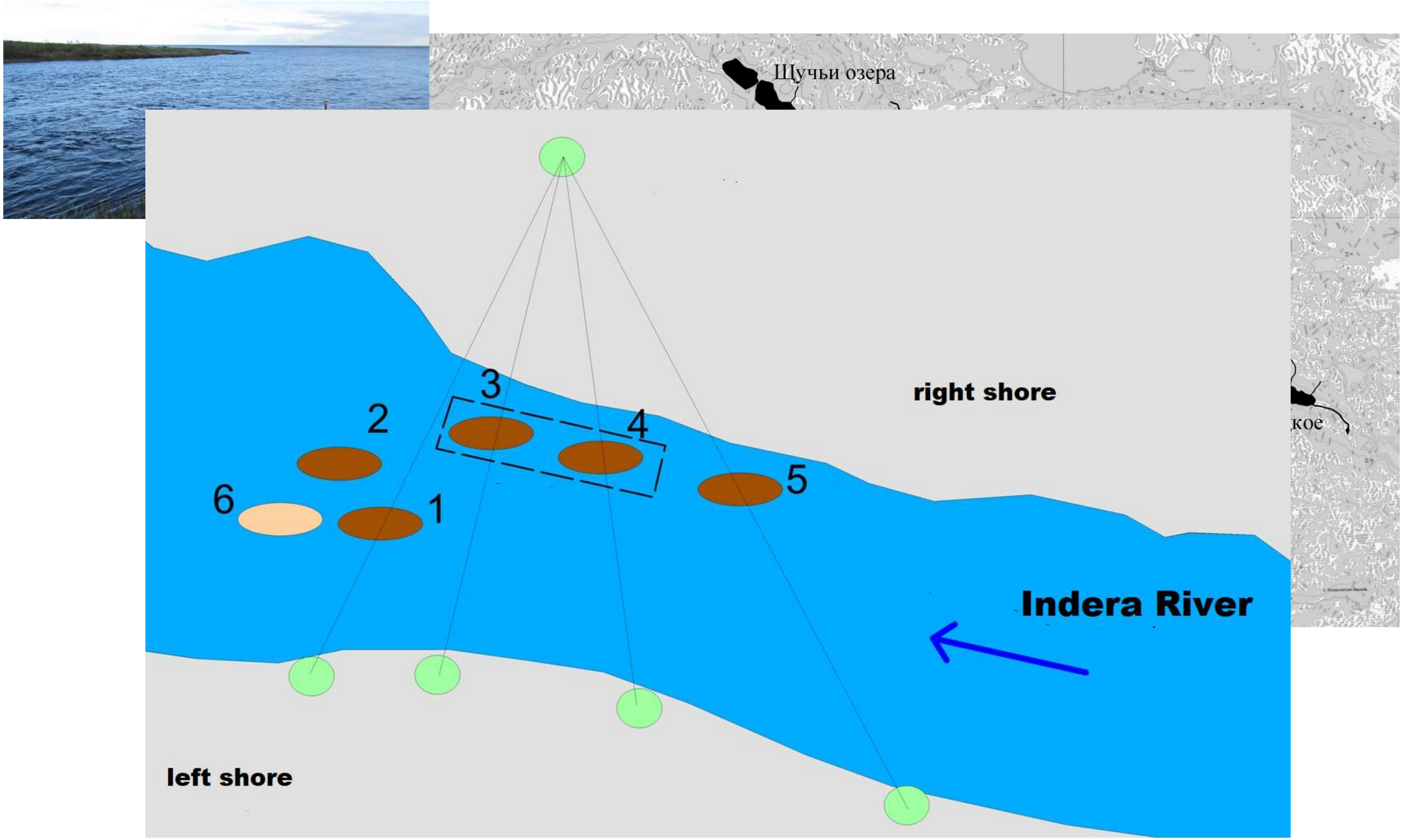
A higher rate of fertilization was observed in the eggs with a larger amount of n-3PUFA, an increased n-3/n-6 ratio (including abundant amounts of PL and a high PL/TAG ratio) and a reduced amount of n-6PUFA (including a lower CHOL/PL ratio).

A reduced rate of fertilization can indicate disturbances in lipid metabolism due to disadvantageous abiotic and biotic conditions during this process and the incomplete maturation of the eggs that developed in different regions of the ovaries of the same female.

	Portions		
2015	Front	Central	Tail
Fatty acids (FAs)			
14:0	2,33±0,12	2,27±0,09	2,28±0,13
16:0	10,85±0,23	10,62±0,19	10,72±0,30
18:0	4,52±0,19	4,43±0,17	4,47±0,16
∑ SFA	19,00±0,43	18,62±0,29	18,83±0,51
16:1(n-7)	7,42±0,35	7,27±0,26	7,32±0,38
18:1(n-9)	23,43±1,13	23,02±0,90	23,14±0,85
18:1(n-7)	4,72±0,19	4,63±0,16	4,66±0,20
20:1(n-9)	1,87±0,29	1,84±0,18	1,87±0,22
∑ MUFA	39,63±1,52	38,91±1,05	39,16±1,3
18:2(n-6)	1,63±0,05	1,63±0,05	1,63±0,06
20:4(n-6)	0,85±0,03	0,89±0,03	0,87±0,05
(n-6) PUFA	3,36±0,09	3,39±0,10	3,36±0,10
18:3(n-3)	1,00±0,05	1,00±0,04	0,98±0,05
18:4(n-3)	0,88±0,08	0,90±0,08	0,87±0,08
20:4(n-3)	2,12±0,11	2,17±0,09	2,12±0,11
20:5(n-3)	14,70±0,90	15,15±0,69	14,94±0,88
22:6(n-3)	13,42±0,85	13,85±0,49	13,77±0,69
(n-3) PUFA	37,21±1,90	38,32±1,16	37,88±1,73
∑ PUFA	41,33±1,90	42,43±1,19	41,97±1,74
∑(n-3)/∑(n-6)	11,12±0,60	11,38±0,40	11,34±0,55
18:3(n-3)/18:2(n-6)	0,61±0,03	0,61±0,03	0,61±0,03
16:0/18:1(n-9)	0,47±0,02	0,47±0,02	0,47±0,02

The Indera River

II. Embryogenesis of pink salmon in nature



Redds of pink salmon: # 6 – eyed stage, hatching and unviable embryos were sampled in October; # 3 and # 4 – pre-larvae were collected in March.

Total lipids and lipid classes in eyed stage, hatching and enviable eggs of pink salmon from the Indera River

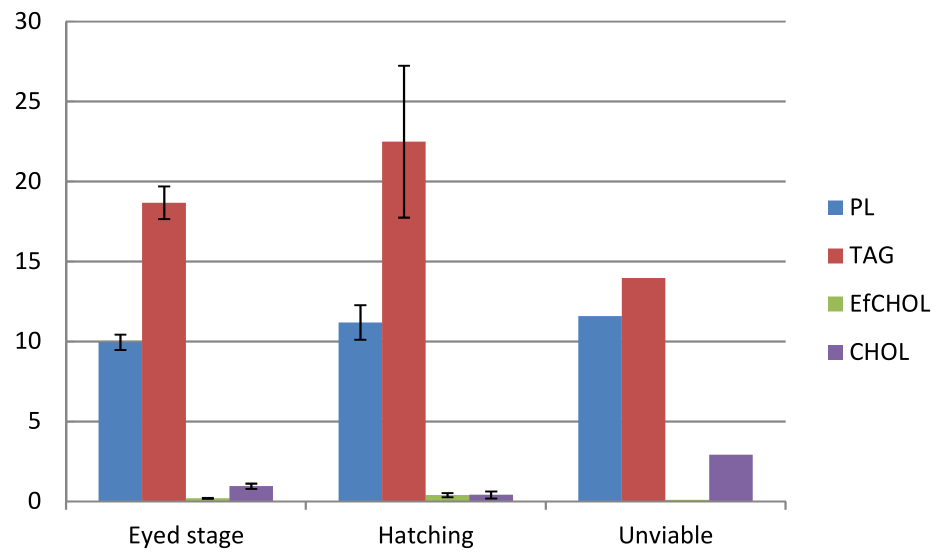
Eyed stage

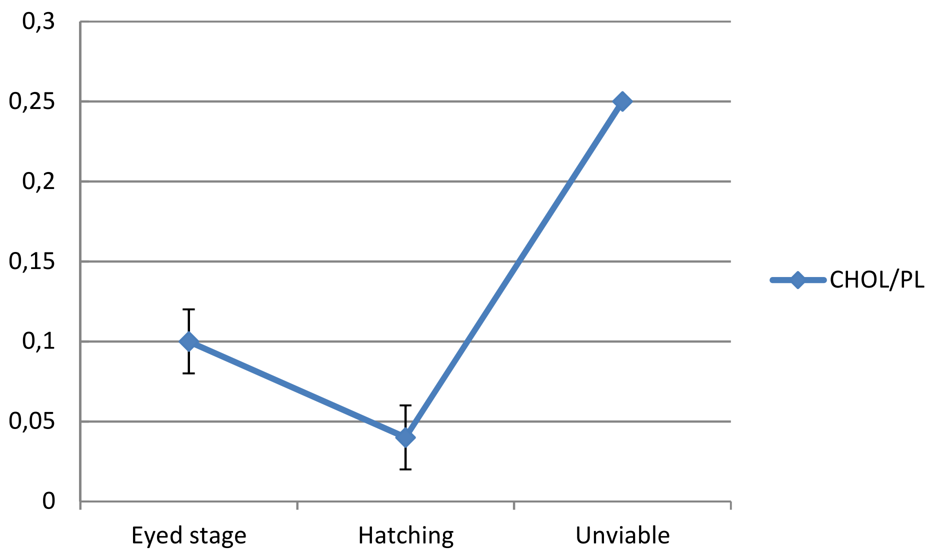
Hatching

Unviable

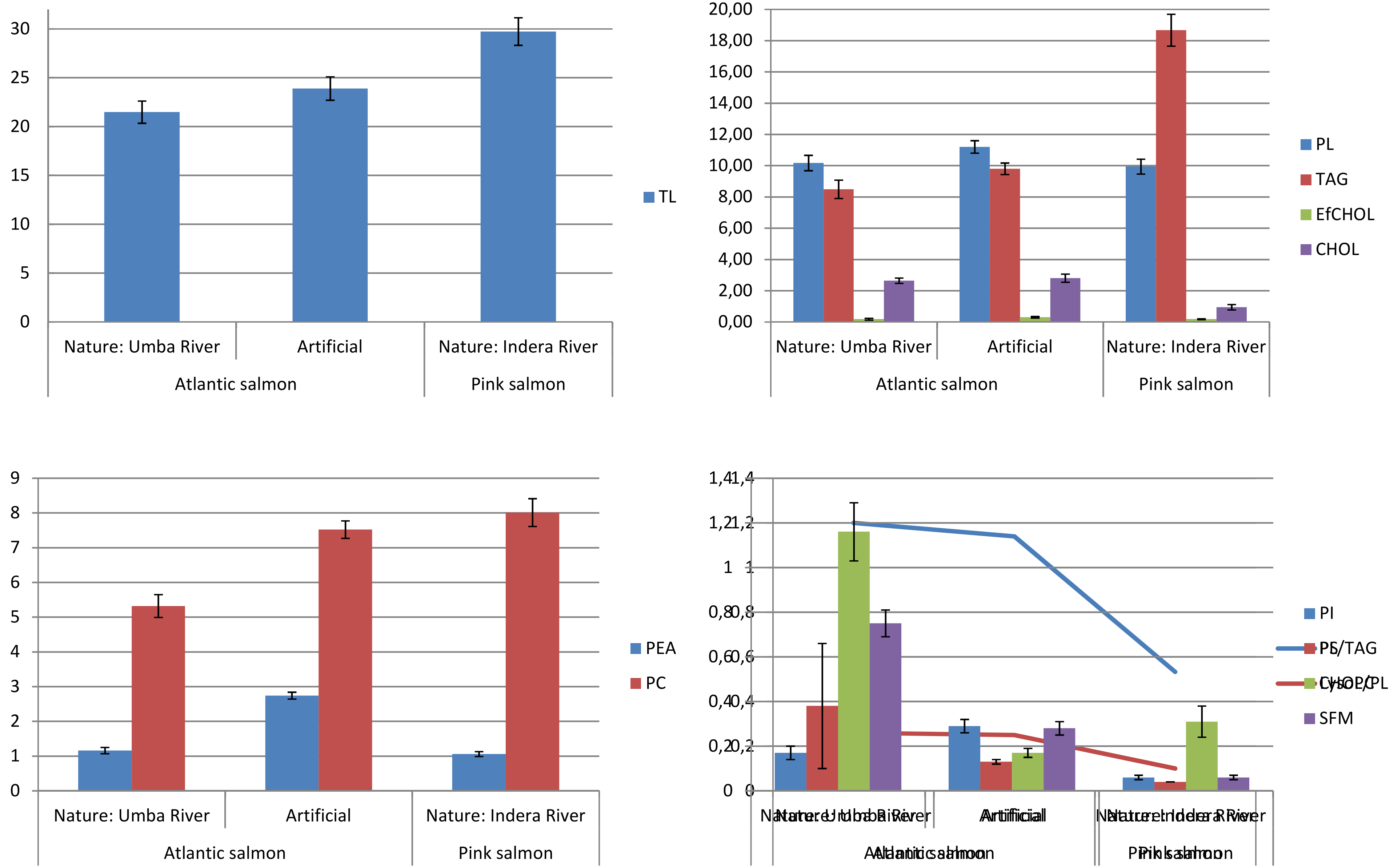
Stage	Eyed stage	Hatching	Unviable
TL (% dry weight)	29,73	34,45	28,51

Lipid profile of eyed stage, hatching and unviable eggs during embryogenesis





Comparative characteristics of the lipid and fatty acid status of eyed-stage Atlantic salmon embryos reared in natural (Umba River) and artificial environments and to pink salmon in nature (Indera River)

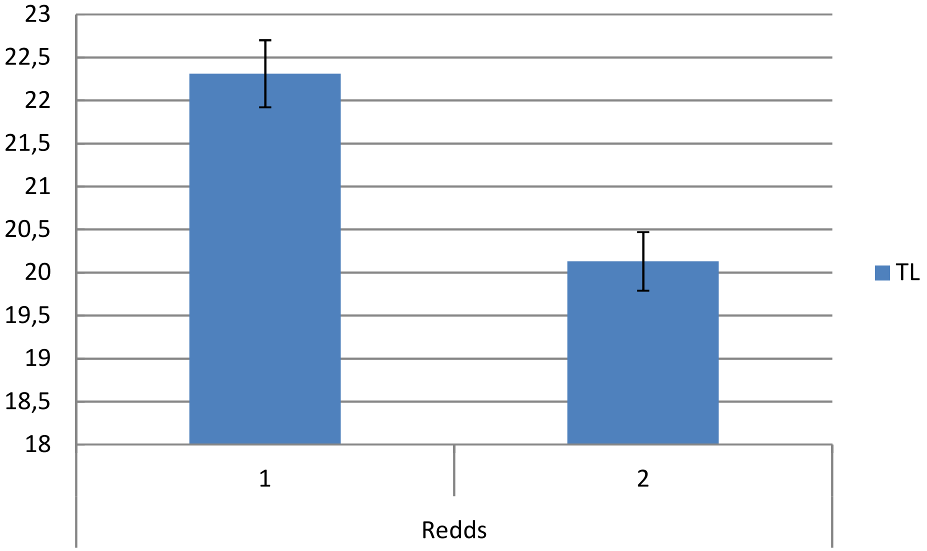


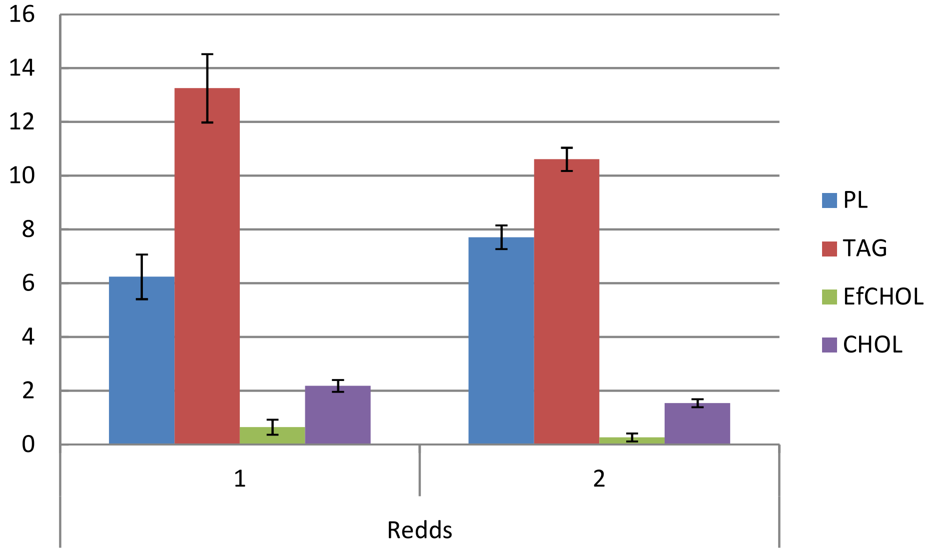
Fatty acids (% sum) profile eyed-stage, hatching and unviable embryos of pink salmon in nature (Indera River)

Indera River, October, t water 1.5 ^{o}C			
FAs/Stage	**Eyed stage**	**Hatching**	**Unviable**
14:0	1,95±0,015	1,92±0,011	1,97
16:0	9,64±0,056	10,73±0,063	10,10
18:0	4,16±0,028	4,47±0,024	4,34
20:0	0,67±0,046	0,57±0,034	0,64
∑ SFA	**16,93±0,093**	**18,22±0,106**	**17,64**
16:1(n-7)	6,52±0,024	6,57±0,207	6,35
16:1(n-5)	0,20±0,002	0,19±0,004	0,21
17:1(n-7)	0,51±0,004	0,48±0,005	0,48
18:1(n-9)	21,08±0,083	22,01±0,027	21,02
18:1(n-7)	4,10±0,014	4,15±0,280	4,23
20:1(n-9)	1,34±0,035	1,46±0,064	1,48
∑ MUFA	**35,04±0,094**	**36,12±0,448**	**35,10**
18:2(n-6)	1,57±0,012	1,46±0,071	1,71
18:3(n-6)	0,04±0,001	0,03±0,004	0,04
20:4(n-6)	0,88±0,004	0,82±0,012	0,81
∑ (n-6) PUFA	3,34±0,020	3,07±0,065	3,53
18:3(n-3)	1,34±0,024	1,17±0,231	1,23
18:4(n-3)	1,15±0,012	0,97±0,163	1,02
20:4(n-3)	3,03±0,030	2,69±0,344	2,81
20:5(n-3)	16,63±0,065	15,72±0,040	15,82
22:5(n-3)	5,07±0,032	5,06±0,222	5,22
22:6(n-3)	16,12±0,053	15,78±0,019	16,33
∑ (n-3) PUFA	**43,92±0,159**	**41,93±0,480**	**42,96**
∑ PUFA	**47,99±0,169**	**45,63±0,549**	**47,23**
(n-3)/(n-6)	13,16±0,079	13,67±0,134	12,17
16:0/18:1(n-9)	0,46±0,002	0,49±0,002	0,48

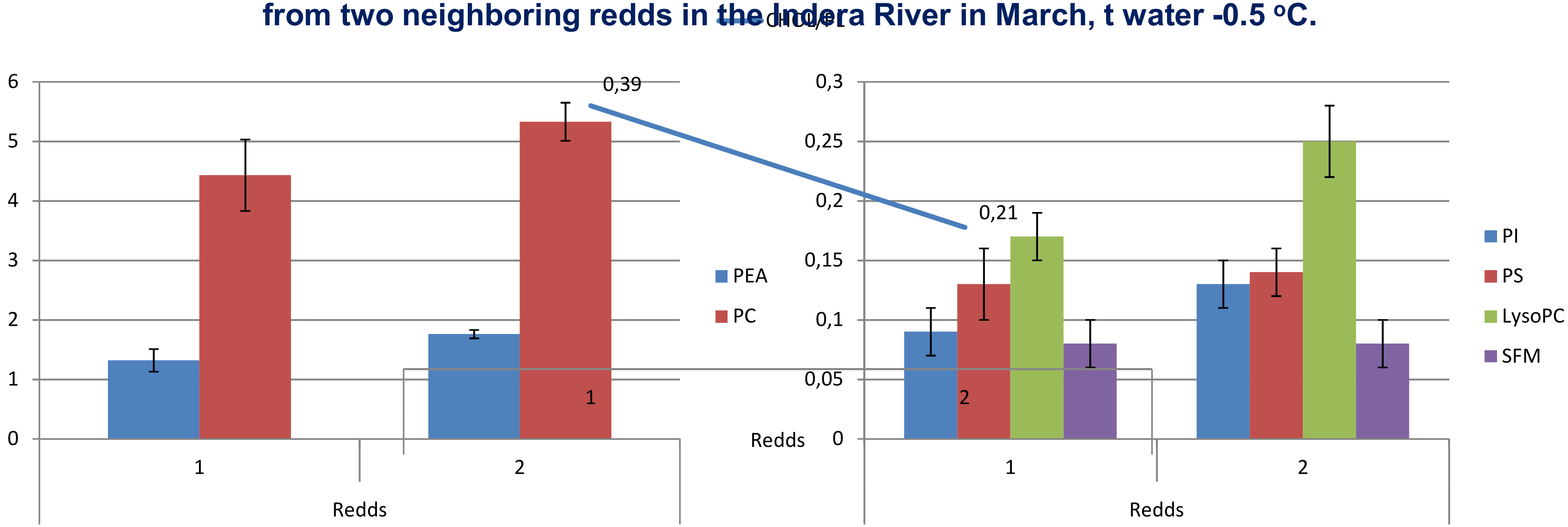
Eyed stage, **Atlantic salmon**		
FAs/Stage	Conditions	
	Umba River t water 0.3 ^{o}C	Artificial T water 4^{o}C
14:0	2.44 ± 0.12	1.35 ± 0.14*
16:0	14.43 ± 0.48	12.30 ± 0.58*
18:0	6.34 ± 0.23	6.32 ± 0.11
Σ SFA	**25.52 ± 0.89**	**22.02 ± 0.94***
16:1(n-7)	5.97 ± 0.23	7.36 ± 0.26*
18:1(n-9)	21.17 ± 0.85	16.65 ± 0.74*
18:1(n-7)	2.25 ± 0.62	4.20 ± 0.51*
Σ MUFA	**33.68 ± 0.71**	**29.96 ± 0.88***
18:2(n-6)	1.47 ± 0.06	2.71 ± 0.06*
20:4(n-6)	0.78 ± 0.05	6.12 ± 0.98*
Σ (n-6) PUFA	3.38 ± 0.28	11.36 ± 0.74*
18:3(n-3)	1.00 ± 0.05	3.52 ± 0.33*
18:4(n-3)	0.54 ± 0.04	0.61 ± 0.02
20:4(n-3)	3.58 ± 0.14	2.90 ± 0.12*
20:5(n-3)	9.12 ± 0.41	7.71 ± 0.40*
22:5(n-3)	5.59 ± 0.27	6.15 ± 0.33*
22:6(n-3)	16.59 ± 0.71	13.66 ±0.45*
Σ (n-3) PUFA	**37.05 ± 1.24**	**35.42 ±1.29***
Σ PUFA	**40.43 ± 1.25**	**46.95 ±1.35***
Σ (n-3)/Σ (n-6) PUFA	16.59	3.88*
18:3(n-3)/18:2(n-6)	0.68	1.3*
16:0/18:1(n-9)	0.68	0.74

Comparative characteristics of the total lipids and lipid classes in prelarvae of pink salmon collected separately from two neighboring redds in the Indera River in March, t water -0.5 ^{o}C.

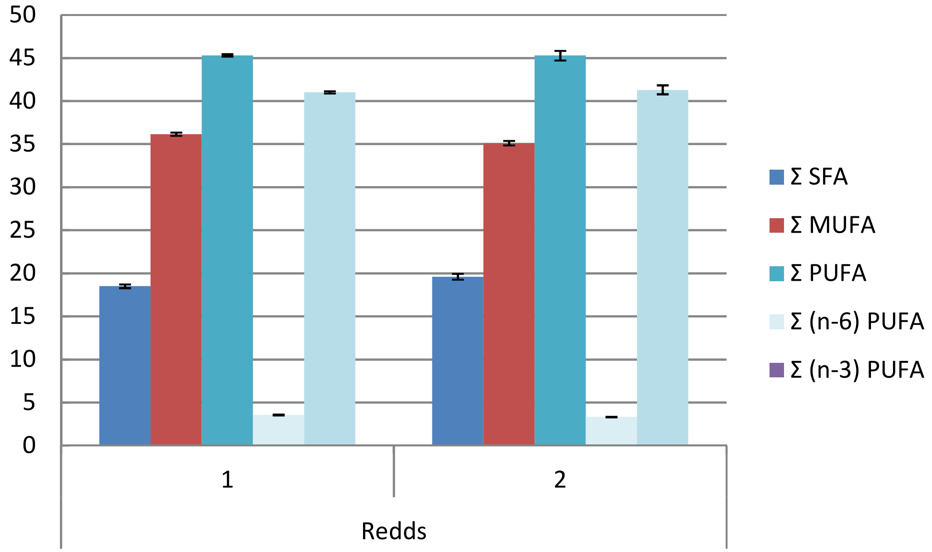


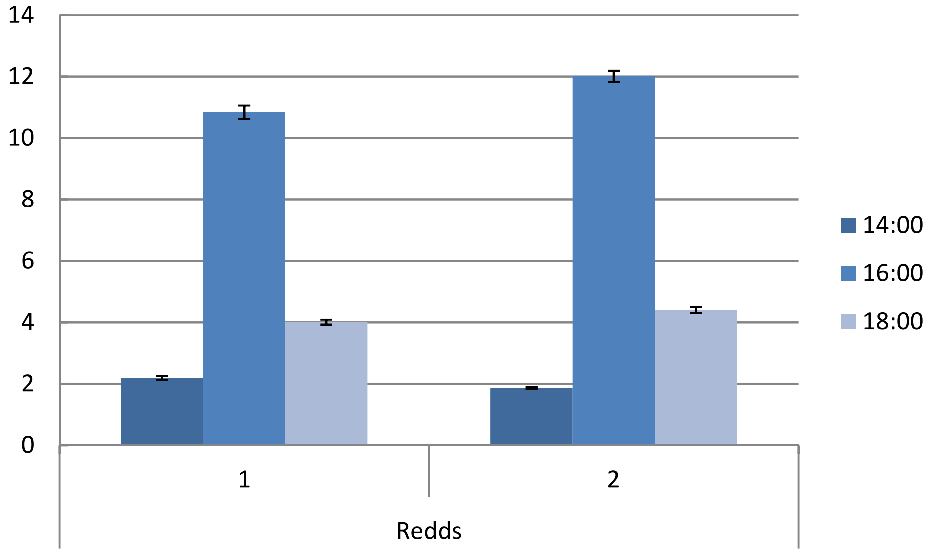


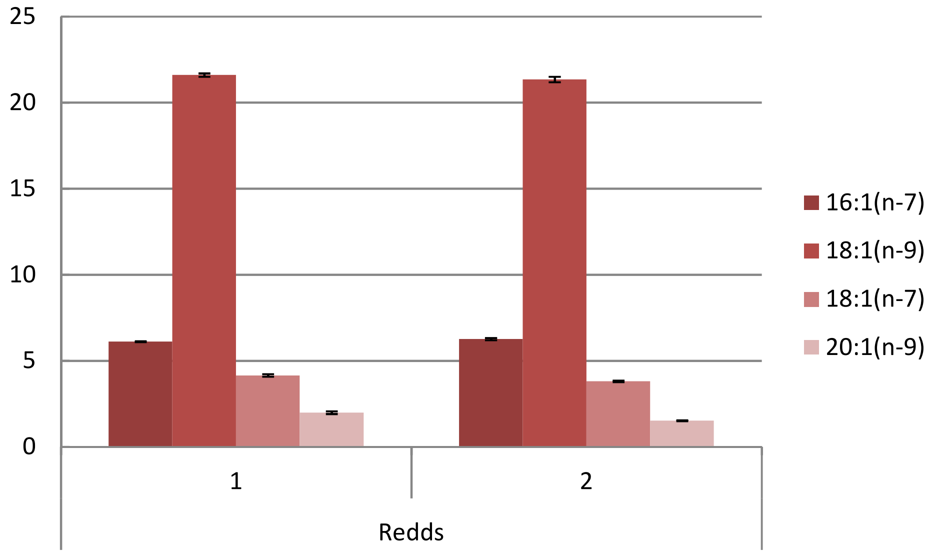
Comparative characteristics of phospholipid classes in prelarvae of pink salmon collected separately from two neighboring redds in the Indera River in March, t water -0.5 ^{o}C.

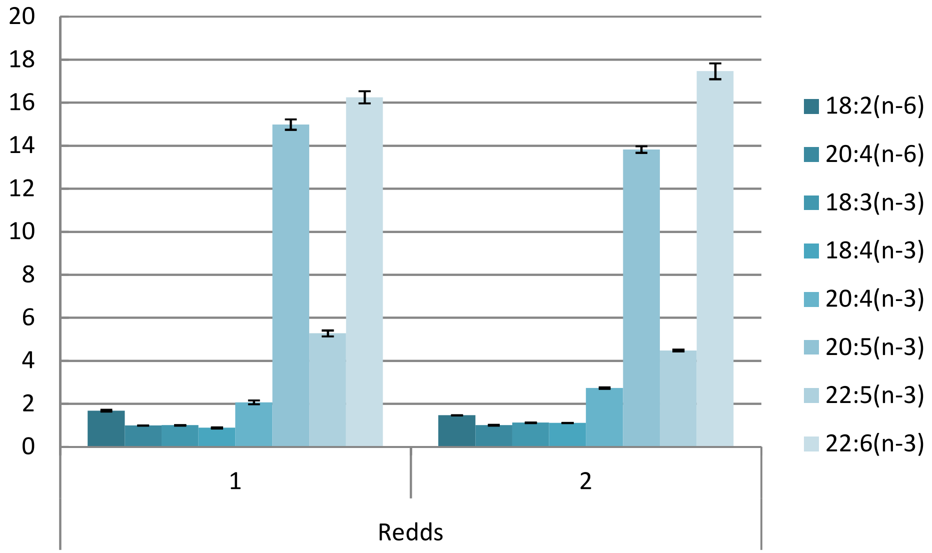


Comparative characteristics of fatty acids in prelarvae of pink salmon collected separately from two neighboring redds in the Indera River in March, t water -0.5 °C.







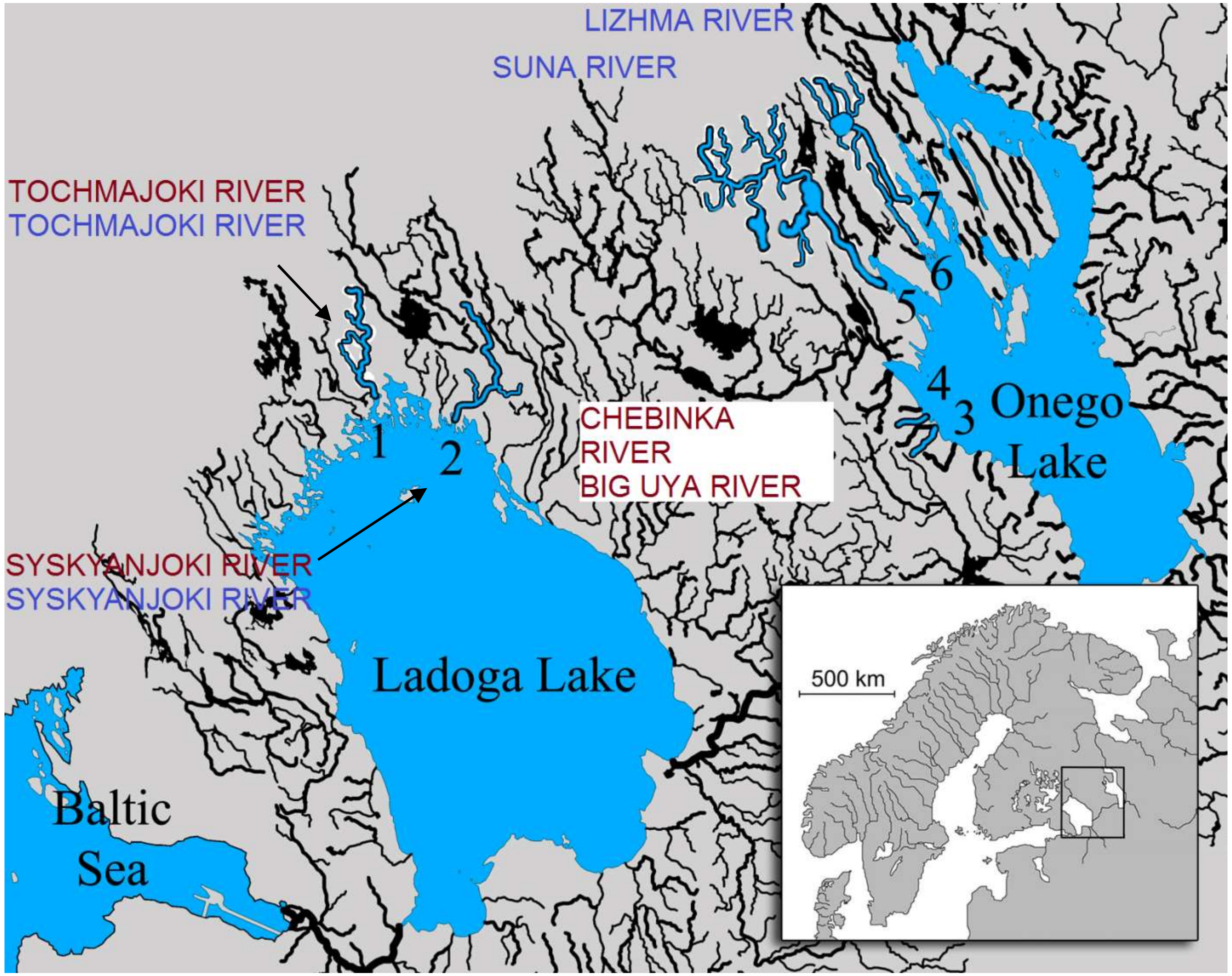


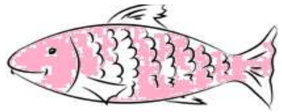
III. Fatty acids profiles of forage objects of salmonids and it's importance in growth and development of young individuals

- The feeding patterns are coupled with the life cycles of major dietary benthic species;
- The condition of the food resources is one of the key factors influencing the survival rates and recruitment of Salminods generations.

The fatty acids composition of rheophilic benthic invertebrate communities (macrozoobenthos) on which juvenile land-locked Atlantic salmon and brown trout forage in rivers with different hydrological characteristics in Onego and Ladoga Lake catchments, and in the Invera River (Kola peninsula) was studied.

AREA OF STUDY: salmonid rivers (Ladoga and Onega Lakes Basins)





BROWN TROUT

ATLANTIC SALMON

LADOGA LAKE BASIN: 1- Tochmajoki River, **2** - Syskyänjoki River

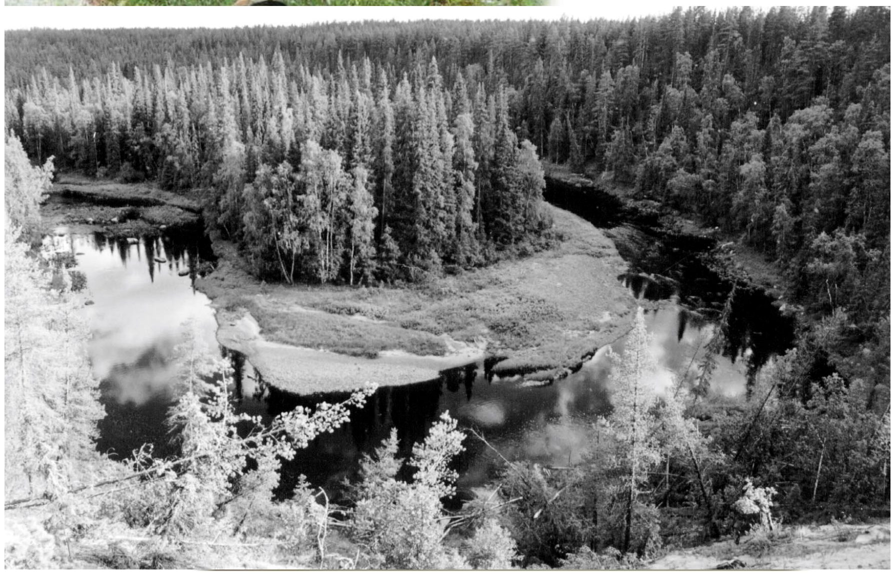
ONEGA LAKE BASIN: 3 – Big Uja River, **4** – Orzega River, **5** – Suna River, **6** – Chebinka River, **7** – Lizhma River

Material and methods:
I. field work

- **Sampling** of aquatic invertebrates was made on typical spawning and nursery grounds of fishes – salmon and brown trout.

pling of macrozoobenthos was made using

frame



<u>“SALMON" rivers - **Suna** River, **Lizhma** River</u>

Larger extent - from 30-50 km

An average annual water discharge in the mouth from 3.5-5.5 m^3/s

A width ranged from 12-15 m to 300-700 m

“Stable” hydrological mode, alternation stretched threshold and the rift of sites

<u>“SALMON-TROUT" rivers - **Syskyänjoki** River, **Tokhmajoki** River</u>

The rivers distinguished by hydrological conditions. are inhabited by juveniles of salmon on the lower thresholds while the juveniles of trout prefer the top thresholds

<u>**Chebinka** River</u>

Water extent - 12-30 km

An average annual water discharge in the mouth from from 0.8 to 3.5 m^3/s

A width ranged from 1.5 to 6-12 m

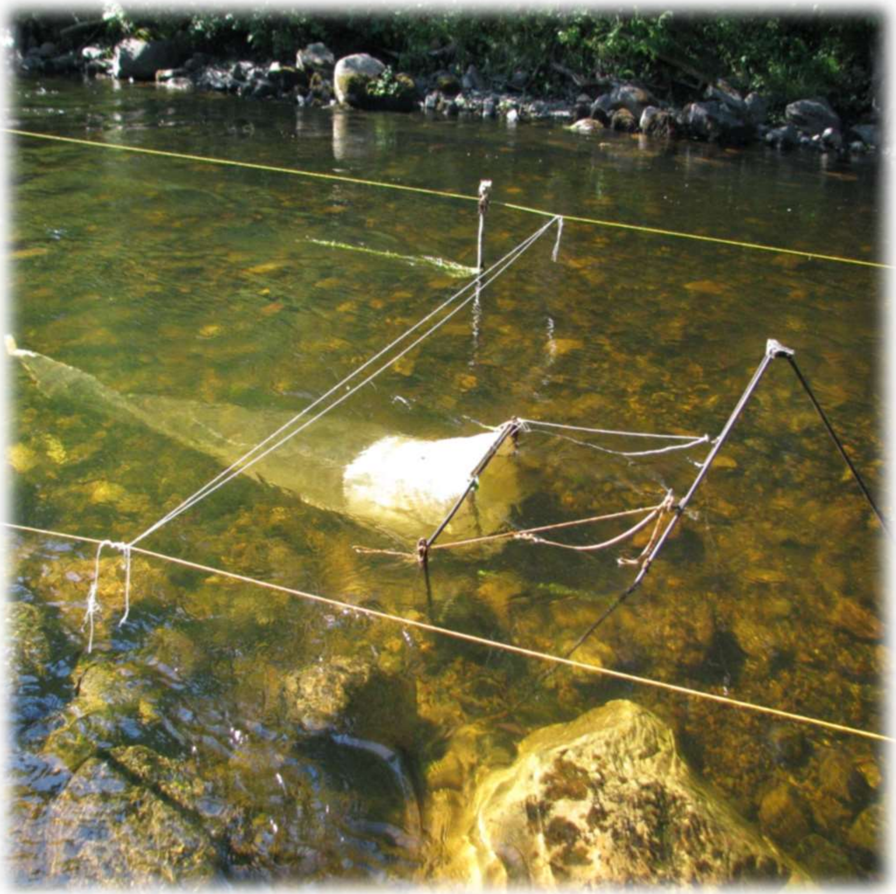
“Unstable” hydrological mode, small thresholds in a bed of river and rifts alternate with holes and short reaches

A water delivery is mainly bound to swamps

pH is 5.2-6.9

Field work:

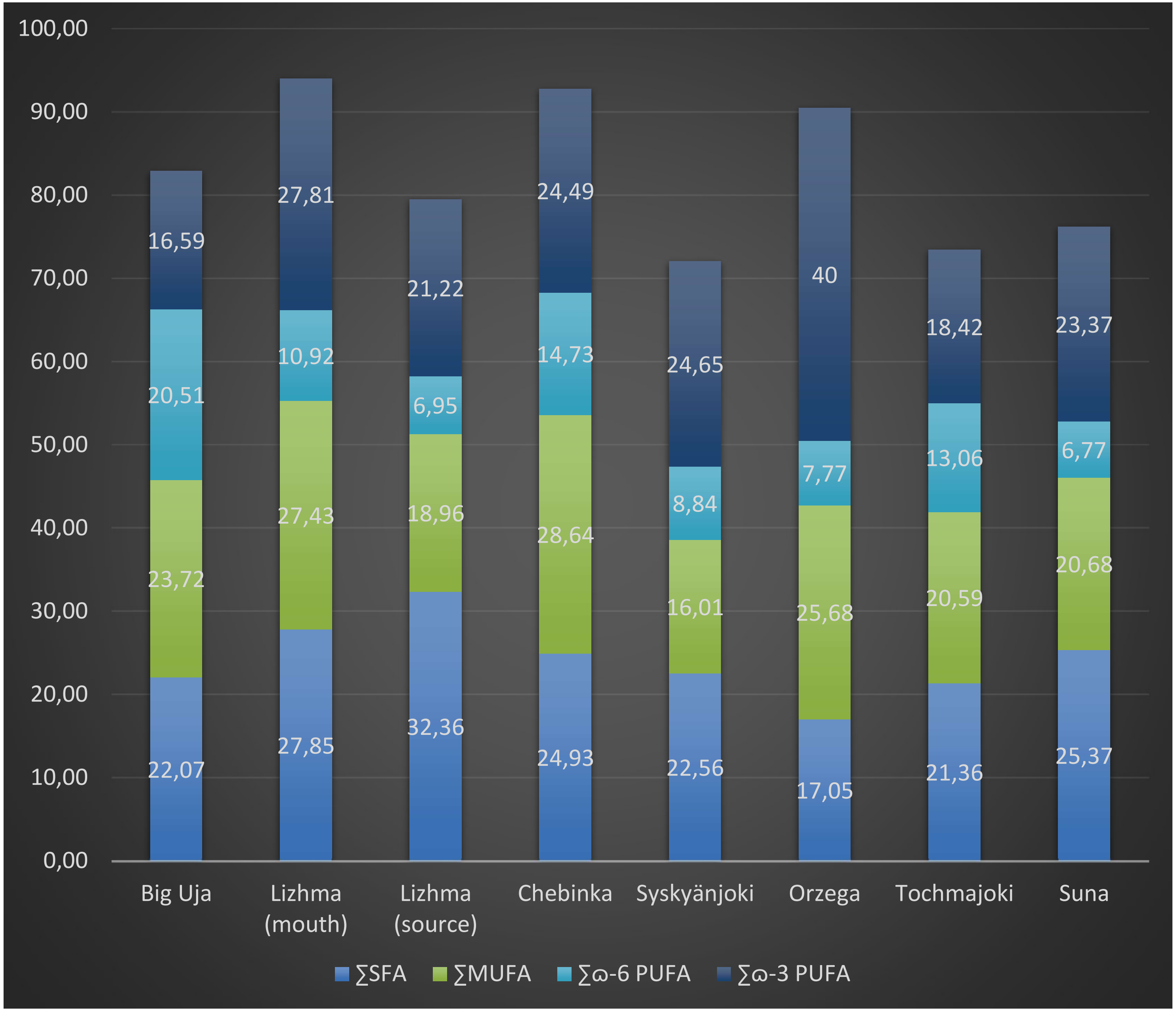
Forage objects of young's salmonids (*Arcynopteryx compacta, Tipulidae , Heptagenia dalecarlica, Arctopsyche ladogensis)* from the Indera River were sampled using drift cage



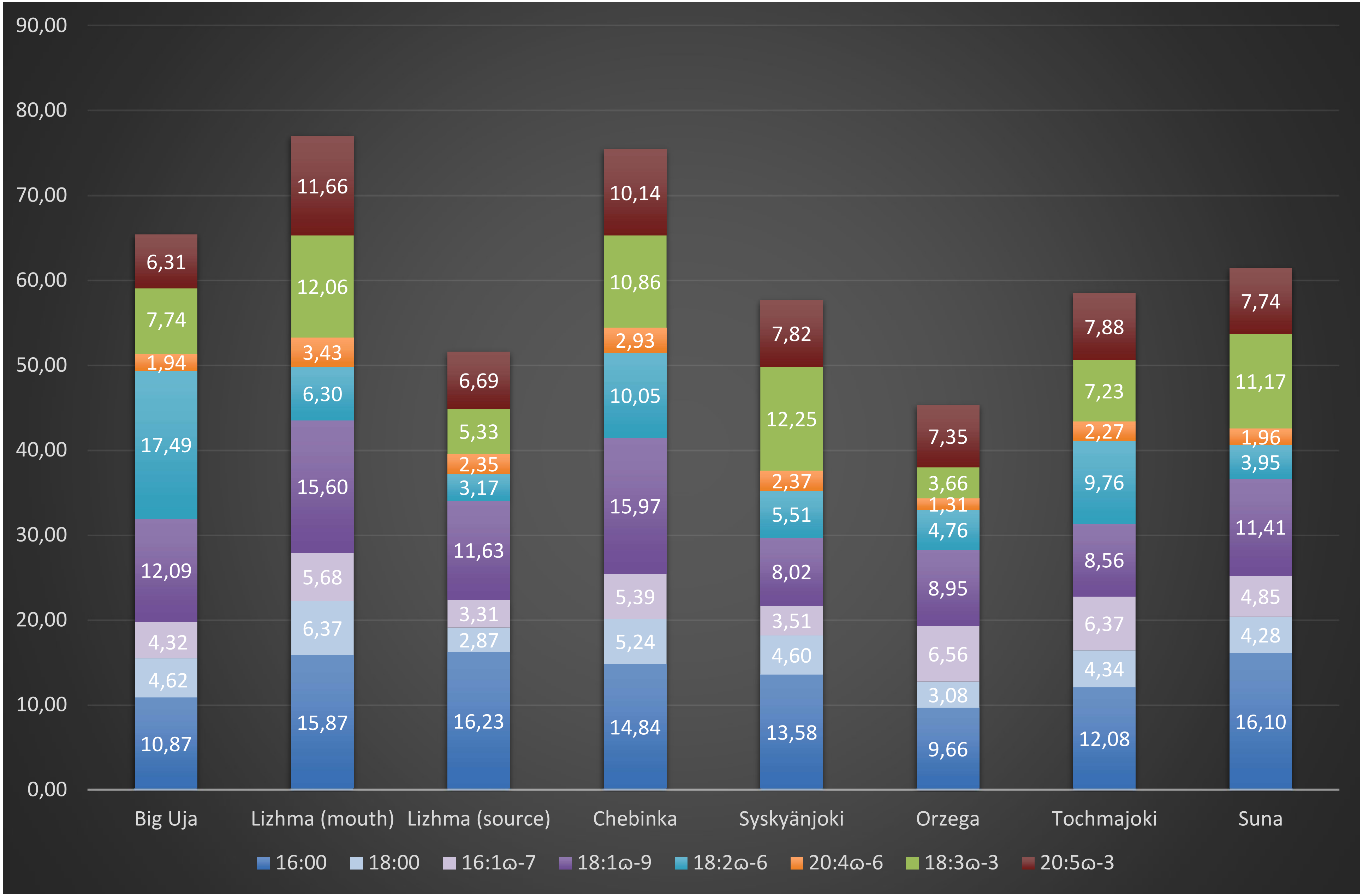
Amount of dominating species of macrozoobenthos in biomass
(*showed in decreasing order*)

Species	Taxa	River							
		Big Uja	Lizhma, mouth	Lizhma, source	Chebinka	Syskyänjoki	Orzega	Tochmajoki	Suna
Hydropsyche pellucidula	Trichoptera	**0.58**	**0.44**	0.94	**0.40**	**0.30**	**0.12**	0.18	**0.67**
Chimarra marginata	Trichoptera	0.00	0.00	0.00	0.00	**0.35**	0.00	0.49	**0.28**
Rhyacophila nubila	Trichoptera	0.00	**0.19**	0.00	**0.29**	0.07	0.08	0.00	0.00
Bivalvia sp.	Bivalvia	0.00	**0.27**	0.02	0.00	**0.22**	0.00	0.02	0.01
Oligochaeta sp.	Oligochaeta	0.00	0.00	0.03	0.00	0.00	**0.35**	0.00	0.00
Tipulidae sp.	Diptera	0.00	0.00	0.00	0.00	0.00	**0.36**	0.00	0.00
Ceratopsyche silfvenii	Trichoptera	**0.21**	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Amount of forage organisms	-	1.00	0.72	0.94	0.99	0.76	**0.28**	0.73	0.97
Shennon index	-	1.23	1.41	0.26	1.61	1.47	**1.48**	1.48	0.82

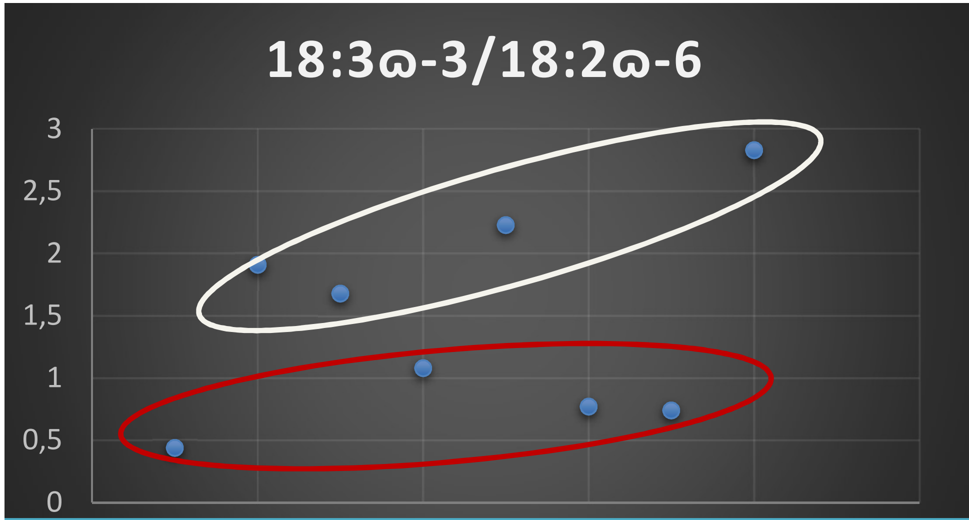
FATTY ACID PROFILES (*PRESENTED BY CLASSES*) OF DOMINATING MACROZOOBENTHOS IN THE RIVERS STUDIED.

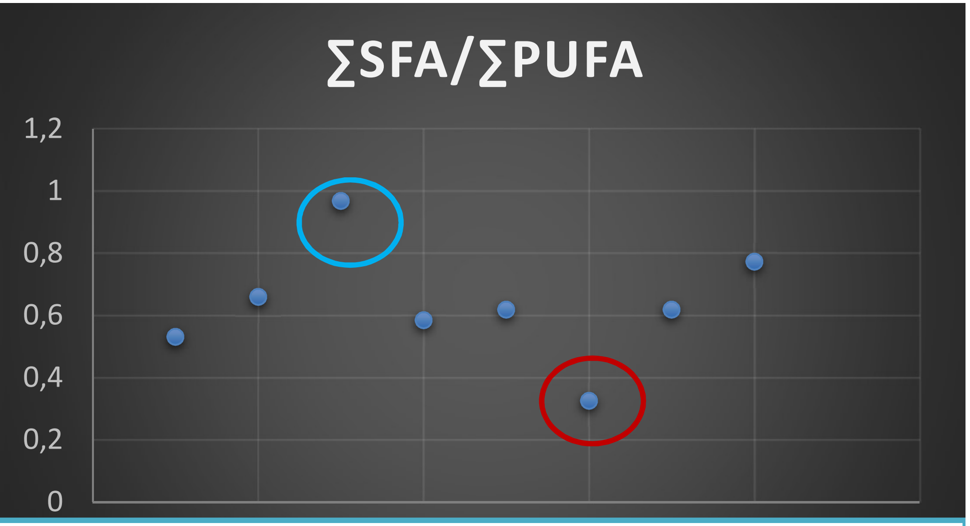


CERTAIN Fatty acids profiles *(% total FA)* in macrozoobenthos



METABOLIC IMPORTANT FATTY ACID RATIOS IN MACROZOOBENTHOS





Big Uja, Lizhma (mouth and source), Chebinka, Suskuanjoki, Orzega, Tokhmajoki, Suna

The indexes of conversions of essential fatty acids into long-chain PUFAs: performed by the ratios 22:6n-3/18:3n-3 and 20:4n-6/18:2n-6 did not vary significantly during growth of juveniles that pointed to the residential brown trout

Young of brown trout (0+, 1+, 2+, 3+) from the ORZEGA river: the level of essential 18:2n-6 and 18:3n-3 fatty increased with age herewith the content of linoleic 18:2n-6 fatty acid was higher

- the structural differences between benthic communities in the rivers studied, in Onego and Ladoga Lake catchments, have a considerable effect on the fatty acid composition of food resources available to juvenile salmonids;
- Quantitative distinctions in FA spectra of zoobenthos from different watercourses are linked to the species specificity of food items;
- the ratio of essential 18:3ω-3/18:2ω-6 FA ratios in zoobenthos from **SALMON** rivers were <u>higher</u> (within 1.68-2.83) than in zoobenthos from **TROUT** rivers (0.44-1.08);
- zoobenthos from **SALMON** rivers had <u>higher</u> SFA/PUFA ratios than that from trout rivers (0.62-0.97 and 0.33-0.62, respectively)

Forage objects from The Indera River

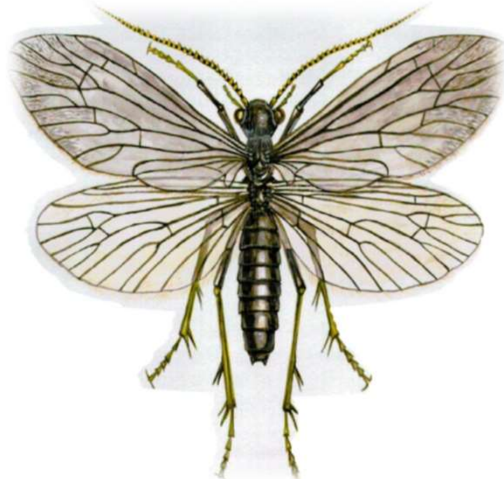
Sample 1: larvae of *Arcynopteryx compacta*

Sample 2: combined

Tipulidae 66%

Arcynopteryx compacta

Heptagenia dalecarlica 24%



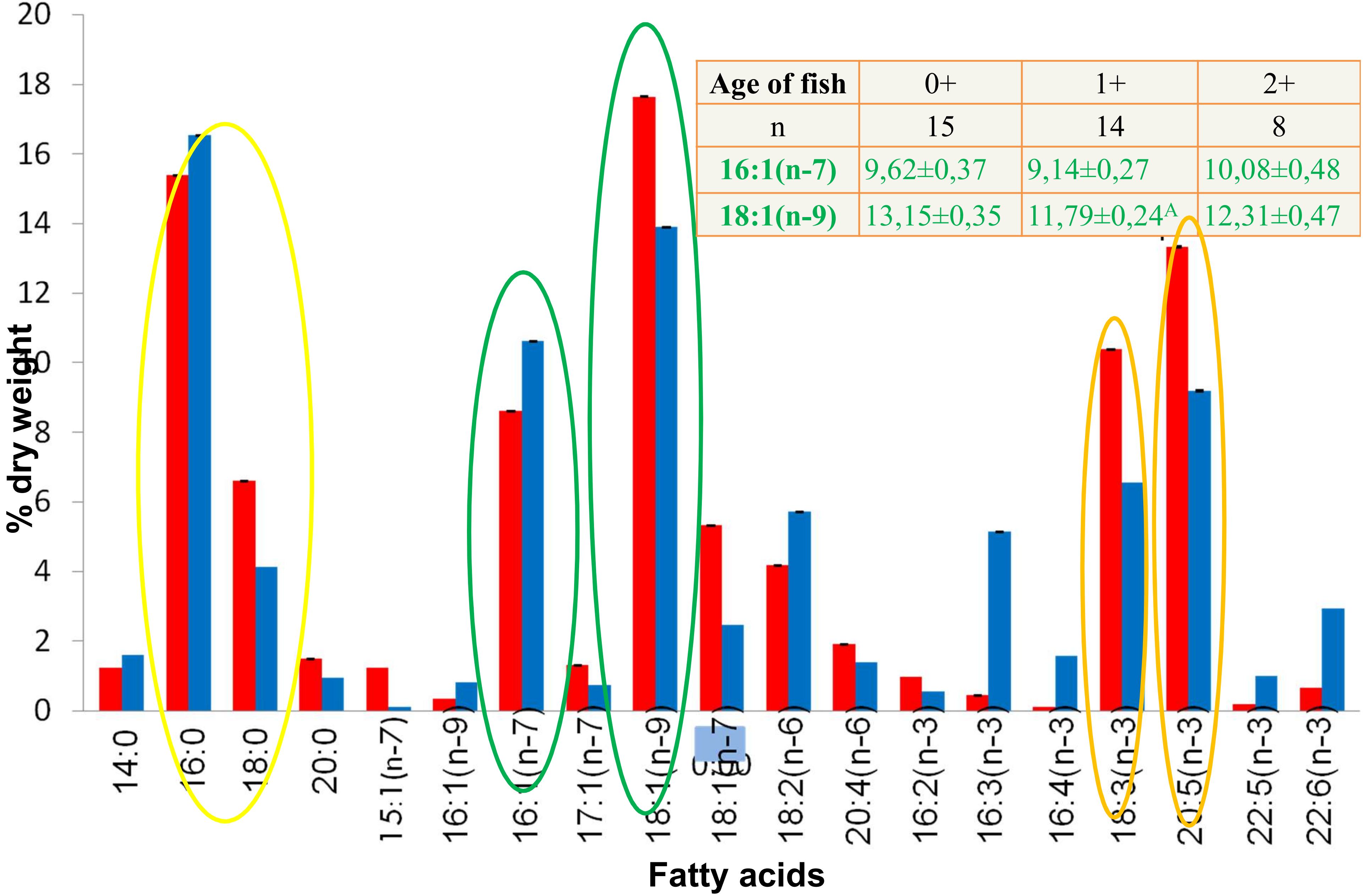
Arctopsyche ladogensis 20%

Fatty acids profile (% sum FA) of forage objects of salmonids in the Indera River

Species	*Arcynopteryx compacta (larvae)*	Combined sample
n	5	5
Σ SFA	25,67±0,014	23,75±0,013*
Σ MUFA	35,98±0,012	34,97±0,029*
Σ Short chained FA	1,14±0,002	0,33±0,0004*
Σ PUFA	37,21±0,021	40,95±0,041*
(n-6) /(n-3)	0,24±0,001	0,32±0,0005*
18:3(n-3)/18:2(n-6)	2,48±0,013	1,15±0,003*
16:0/18:1(n-9)	0,87±0,001	1,19±0,001*

* – *($p \leq 0,05$).*

Fatty acids spectrum of dominating forage objects of juveniles from the Indera River



Age of fish	0+	1+	2+
n	15	14	8
16:1(n-7)	9,62±0,37	9,14±0,27	10,08±0,48
18:1(n-9)	13,15±0,35	11,79±0,24[A]	12,31±0,47

THANK YOU FOR YOUR ATTENTION!