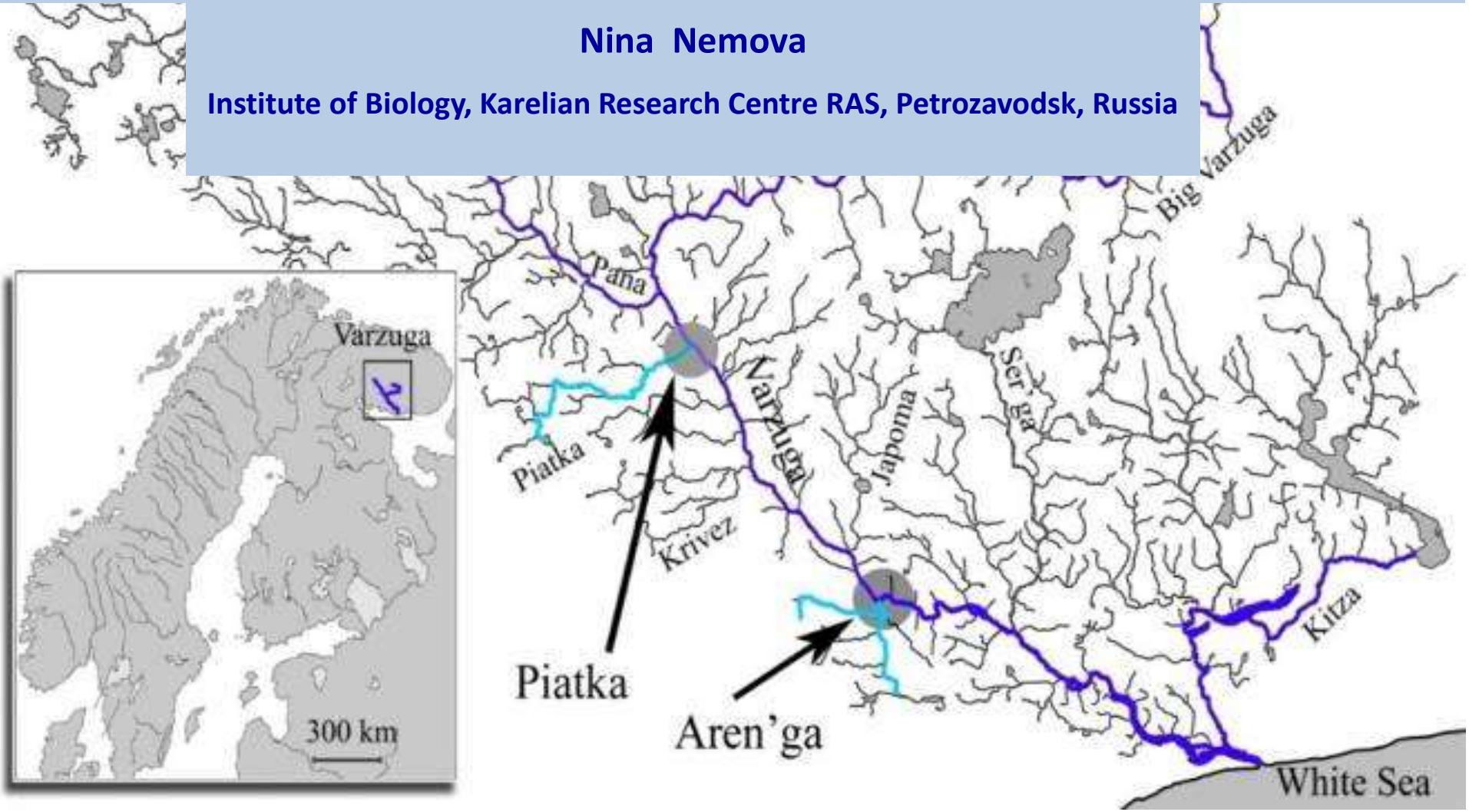
BIOCHEMICAL CHANGES IN THE EARLY DEVELOPMENT OF SALMONID FISH IN THE KOLA PENINSULA

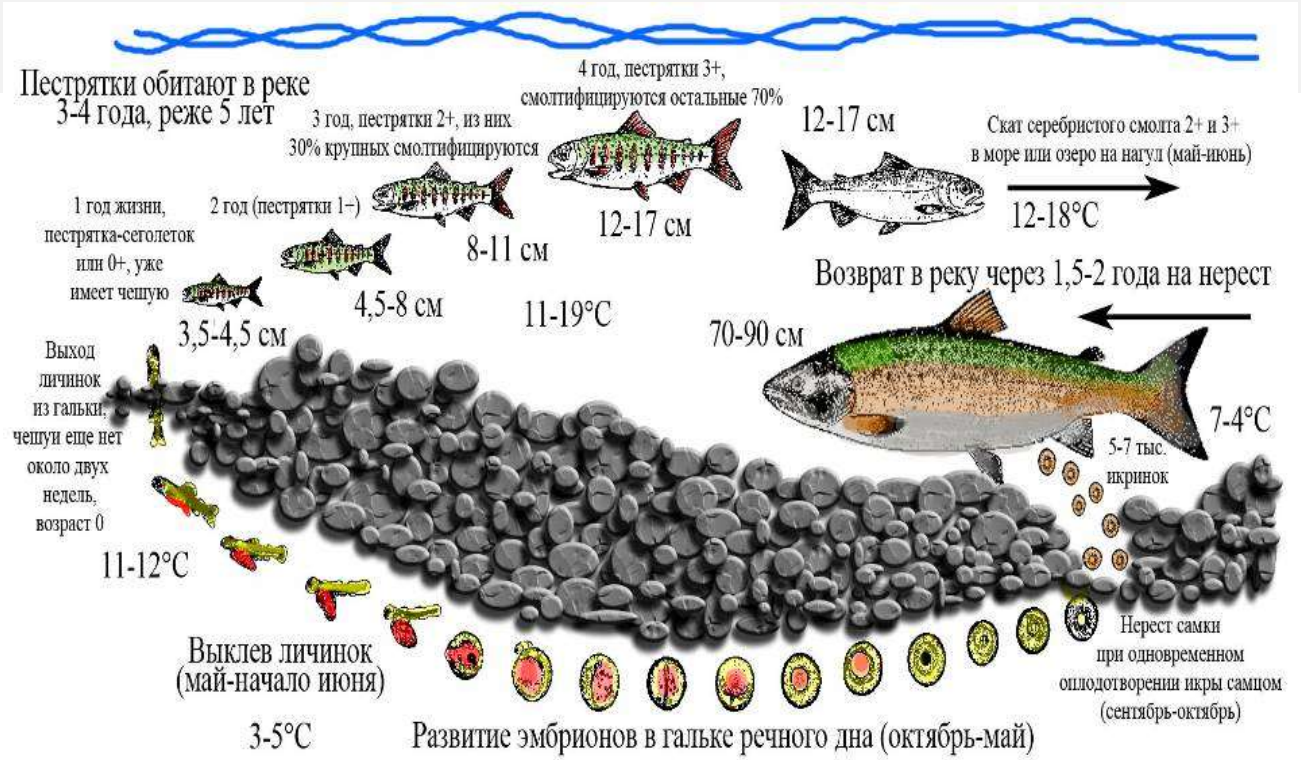
Nina Nemova

Institute of Biology, Karelian Research Centre RAS, Petrozavodsk, Russia



The conditions of life in the river are extremely important for the replenishment of the Salmonid population because the high mortality of fish at this period of life is known

Life cycle of Atlantic salmon in the river

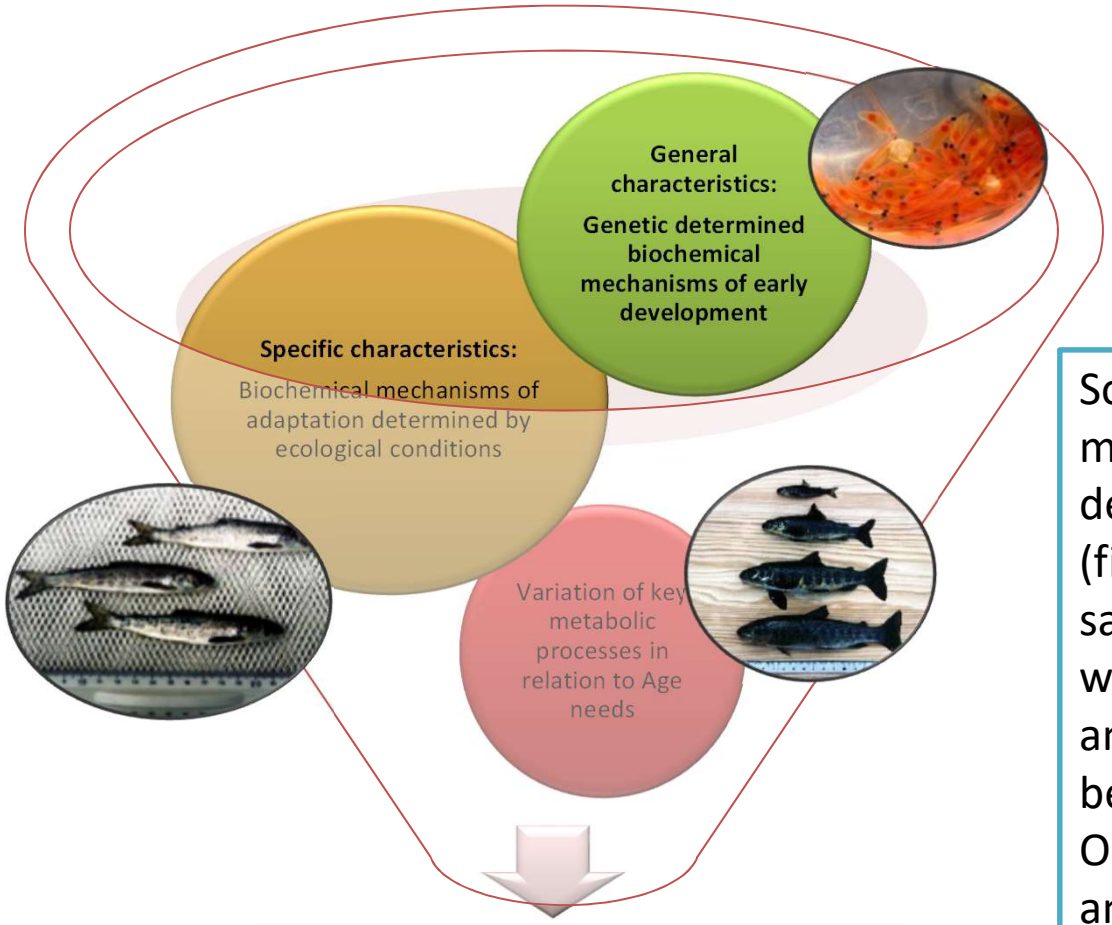


By Veselov A.E.

Ontogenesis of Salmonids that is realized in the river are characterized by: significant morphological and functional transformations associated with fundamental modification of cellular metabolism, changes in the rate and interconnection among different pathways of metabolism

Russian Science Foundation

***«Salmonids of the northwest of Russia: ecological and biochemical mechanisms of early development»* 2014-2018**



Differentiation of juveniles

Migration of smolts to the sea – only if an individual is preperaed on biochemical level – adaptations and alterations on biochemical status to new environment

Life strategy choose

Resident/Anadromous

Some of the 100 biochemical metabolism indicators of early development processes of the juvenile (fingerlings, pre-parr, parr, smolts) salmonids at 0+, 1+, 2+, 3+, 4+ ages were collected from different biotopes and microbiotopes of Salmonid rivers belonging to the White Sea Basin, Onega and Ladoga Lakes Basins were analyzed

- ***Salmo salar* L.**
- ***Salmo trutta* L.**
- ***Oncorhynchus gorbuscha* Walb.**
- **Macrozoobenthos (food objects for juvenile)**

The system of biochemical metabolism indicators includes parameters of :

Lipids:**Total lipids, TAG, PL, PI, PS, PEA, PH, LPH, SM cholesterol, ethers cholesterol, Fatty acids (Total, PUFA, MUFA), ratio** *of CHOL/PL and TAG/PL, EFA/PUFA, 16:0/18:1(n-9), 18:2n-6/18:3n-3, 22:6n-3/18:3n-3, 20:4n-6/18:2n-6 ets.*

Enzymes of energetic and carbohydrate metabolism: COX, *EC 1.9.3.1*, MDH, *EC 1.1.1.37*, LDH, *EC 1.1.1.27*, G-6-PDH, *EC 1.1.1.49*, 1-GPDH, *EC1.1.1.8*., aldolase *EC 4.1.2.13*

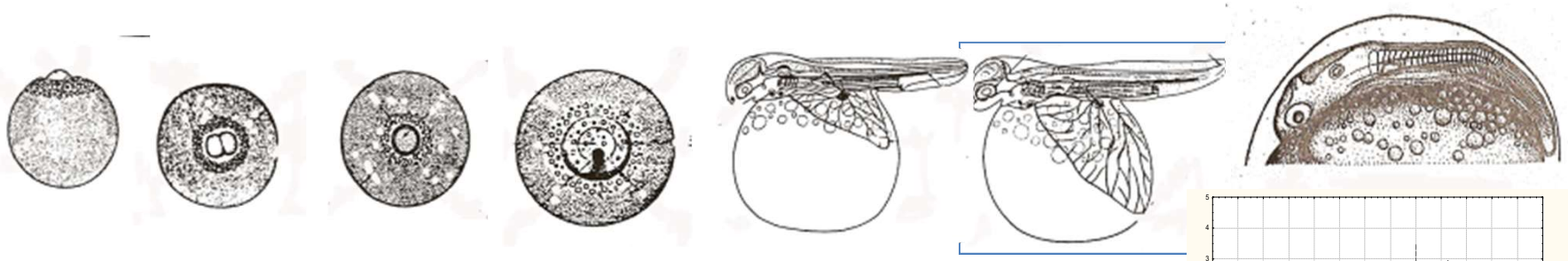
Proteolysis enzymes: μ- and m-calpains *EC 3.4.22.52* and *EC 3.4.22.53*, lysosomal proteinases *CatB*, *EC 3.4.22.1*, *CatD*, *EC 3.4.23.5*, proteasome, *EC 3.4.25.1,* collagenesis *EC 3.4.24.3*, free hydroxyproline, protein-bound hydroxyproline

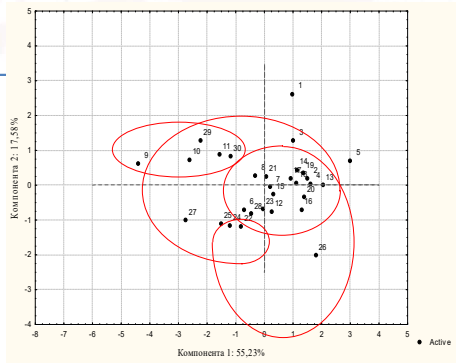
Molecular genetic indicators of expression of genes of myosin heavy chain, MyoD, Myf-5, myogenin and COX, protein content

Scheme of the presentation

- **Biochemical differentiation salmonids during embryogenesis**
- **Biochemical differentiation of juvenile salmonids**
- **Biochemical heterogeneity of fingerlings Atlantic salmon inhabited different biotopes of the Varzuga River**
- **Pink salmon *Oncorhynchus gorbusha* L.**

Biochemical differentiation salmonids during embryogenesis





Biochemical differentiation of salmonids appears already during embryogenesis

the most explicitly for structural lipids as **PI, PS, PEA, PH, LPH, SM,** and certain fatty acids and their indexes – Σ**SFA** (due to 14:0 and 16:0), Σ**MUFA** (due to 18:1(n-9) and Σ**PUFA** (n-3) (20:4(n-3), 20:5(n-3) and 22:6(n-3)), ratio **16:0/18:1(n-9)** (lipid metabolism intensity), amount of **18:1(n-9),** activity **COX, MDH, 1-GPDH & G-6-PDH, calpains**

As a result, in the process of distribution from spawning nests a part of hatched
larvae of salmon have certain advantages that enable it to inhabit actively the best nursery areas

COMPARATIVE LIPID AND FATTY ACIDS STATUS OF ATLANTIC SALMON DURING EMBRYOGENESIS AT EYE PIGMENTED STAGE IN NATURE AND ARTIFICIAL CONDITIONS

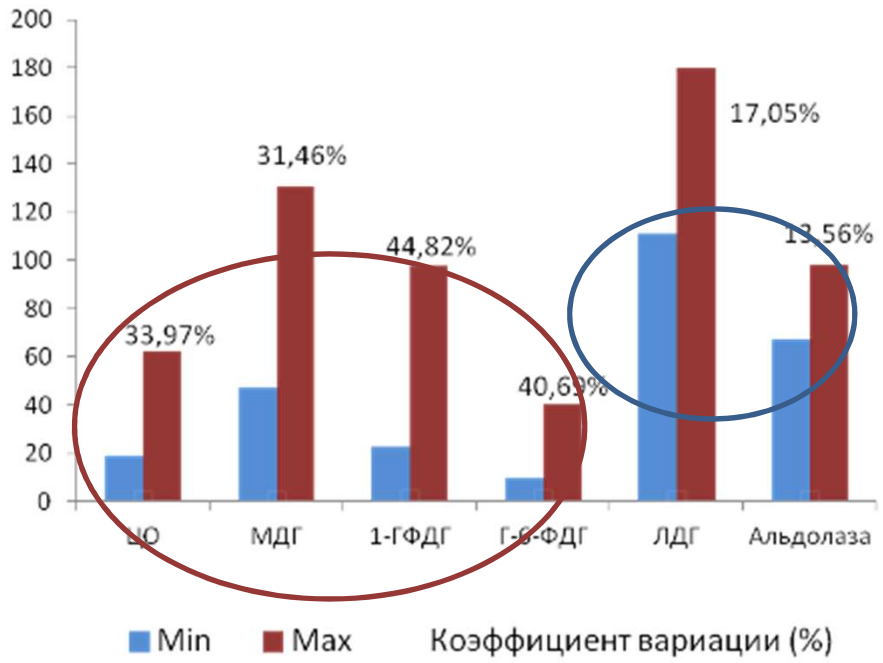
% dry weight	Conditions	
	Umba River **0.2 – 0.3°C**	Artificial **4°C**
Total lipids	21.48 ± 1.14	23.9 ± 1.2*
Sum PL – structural lipids	10.17 ± 0.49	11.2 ± 0.4*
PI	0.17 ± 0.03	0.29 ± 0.03*
PS	0.38 ± 0.28	0.13 ± 0.01*
PEA	1.16 ± 0.09	2.74 ± 0.1*
PC	5.32 ± 0.33	7.52 ± 0.25*
LysoPC	1.16 ± 0.13	0.17 ± 0.02*
SFM	0.75 ± 0.06	0.28 ± 0.03*
TAGs – energetic lipids	8.49 ± 0.59	9.8 ± 0.37*
Cholesterol esters	0.18 ± 0.06	0.3 ± 0.05*
Cholesterol	2.64 ± 0.17	2.8 ± 0.26*
PL/TAG	1.20	1.14*
CHOL/PL	0.26	0.25
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
20:4(n-6)/18:2(n-6)	0.5	2.3*
22:6(n-3)/18:3(n-3)	16.59	3.88*
20:4(n-6)/20:5(n-3)	0.09	0.79*

• Constant level of TL and certain lipid classes (PL, TAG, CHOL, EfCHOL) can be explained by essential role of lipids in genetically determined program of embryogenesis and their importance as structural and energetic components

• Differences were found for some PL fractions – higher level of PI, PEA, PC and lower level – of PS, LysoPC, SFM, and in fatty acids - Sum SFA (due to 14:0 and 16:0), Sum MUFA (due to 18:1(n-9) and Sum PUFA in (n-3) family (due to 20:4(n-3), 20:5(n-3) and 22:6(n-3)), ratio of intensity of lipid metabolism 16:0/18:1(n-9), amount of physiologically important 18:1(n-9)

Energy and carbohydrate metabolism enzymes in Atlantic salmon Salmo salar L. embryos (eyed egg phase), n=20

COX, MDH, 1-GPDH, G-6-PDH, LDH, aldolase



Min and Max activity of enzymes in samples of embryos (mkmol/min/g) and variation coefficient (%)

During salmon embryo development carbohydrates are used not only as an energy source but also for the synthesis of structural components

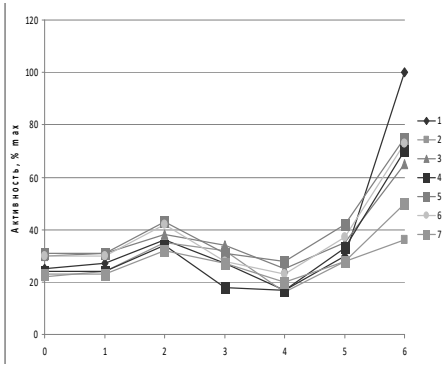
***The qualitative differentiation is manifested in two directions (strategies)**:*

- some individuals have a high level of energy metabolism and synthesis of glycerophosphate (precursor of structural and storage lipids) but a low level of the pentose phosphate pathway (PPP),

- whereas others demonstrate a lower energy metabolism and glycerophosphate synthesis simultaneously with a high level of oxidation of carbohydrates via PPP.

Activity of proteolytic enzymes and protein content in at different stages of salmon embryo development (significant differences ($p \leq 0.05$) relative to stage 0 (before fertilization)

	No. Developmental stage	Activity calpains	Activity cathepsina B	Protein content (1 spawn)
0	Eggs before fertilization	**1.62 ± 0.10**	2.91 ± 0.15	0.45±0.03
1	Blastodisc formation	1.0 ± 0.07*	2.90 ± 0.15	0.39±0.02*
2	Cleavage (early gastrula)	**1.30 ± 0.08***	**4.91 ± 0.30***	0.56±0.04*
3	Tailbud formation	0.78 ± 0.05	3.21 ± 0.25	0.45±0.03
4	Beginning of heart tube pulsation and circulation	0.60 ± 0.05*	2.50 ± 0.20	0.57±0.04*
5	Eye pigmentation	**1.20 ± 0.10***	**6.52 ± 0.55***	0.58±0.04*
6	**Hatching**	0.7 ± 0.05*	0.72 ± 0.06*	0.98±0.07*



aminopeptidases

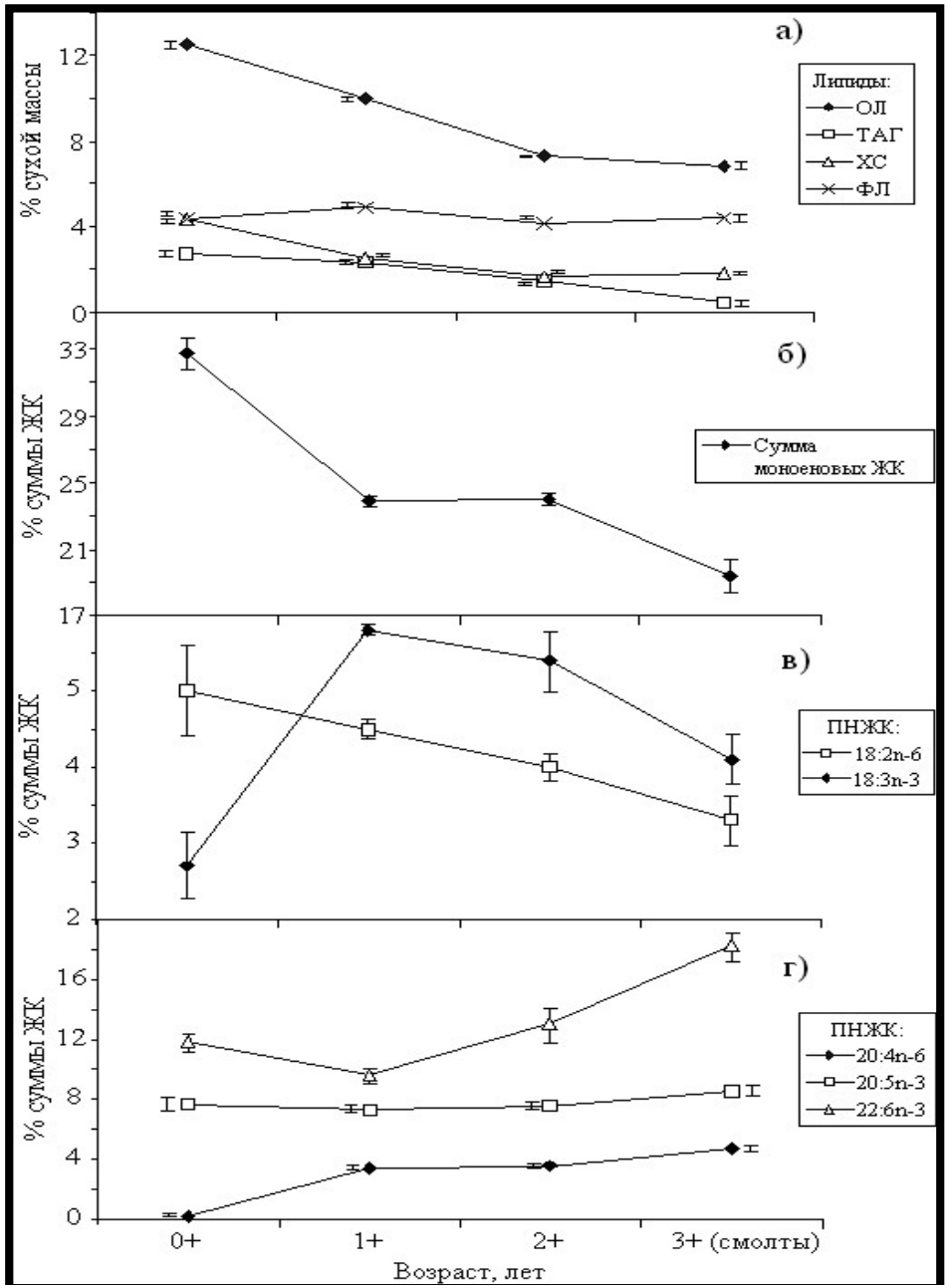
A synergistic activation of cysteine-dependent proteases of different cellular compartments—lysosomal cathepsin B and cytosolic calpains—was detected in the embryonic development of salmon at the gastrulation stage, when reserve yolk proteins are actively used for biosynthesis and building of protein structures of the embryo

Biochemical differentiation of juvenile salmonids

The juvenile of 0+ and 1+ ages stand out from other age groups studied by indices of metabolism

At this period juvenile of salmon are at the stage of choosing a life strategy

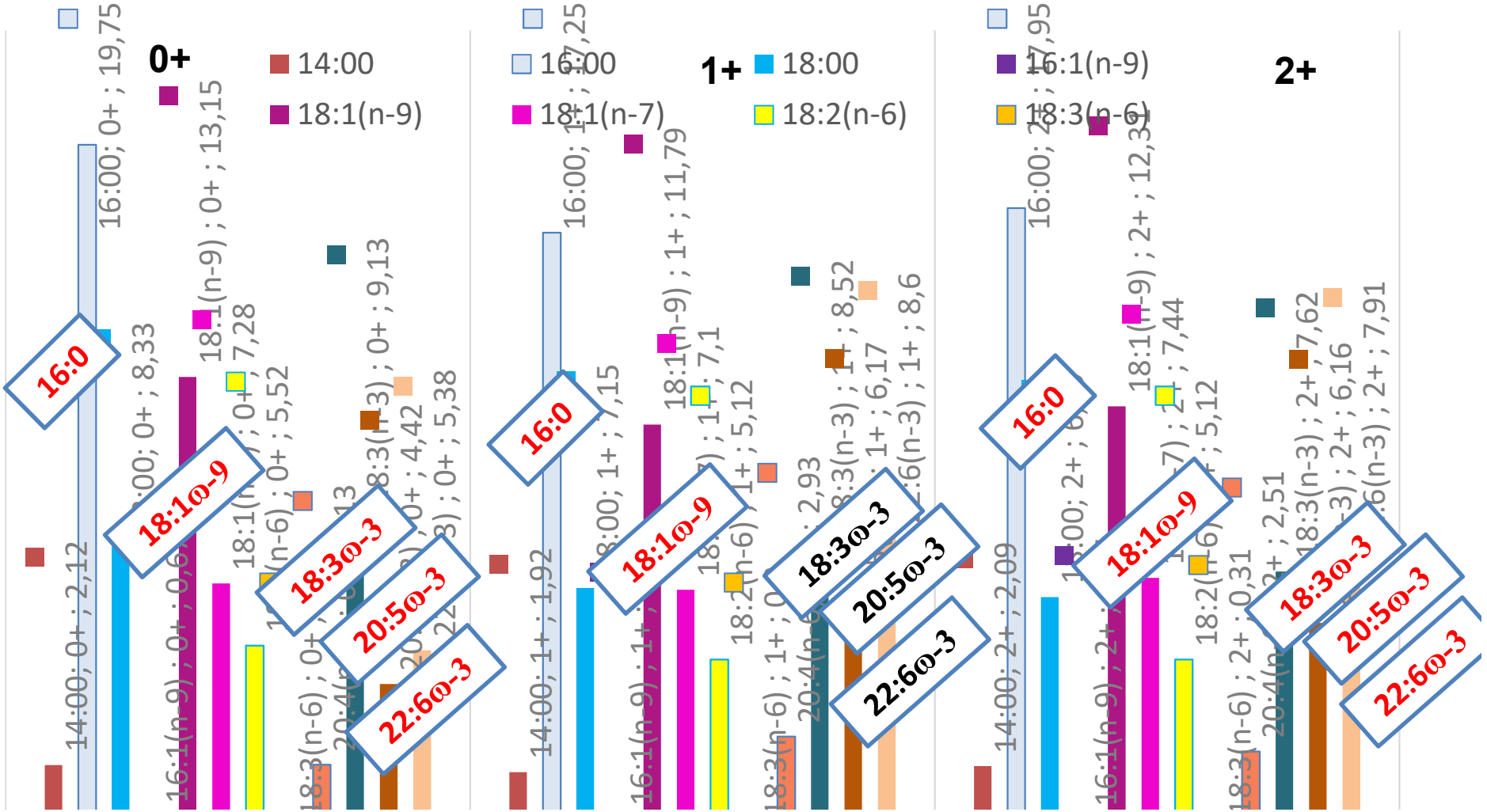
Age-related changes in the lipid and FA status of juvenile Atlantic salmon (Varzuga)



Age-related changes in the lipid status of juvenile Atlantic salmon are a rise of **PUFA – 20:4n-6, 20:5n-3 and 22:6n-3**, and a reduction of energy-intensive **TAG**, as well as **cholesterol (CHOL), cholesterol esters (CE)** and **total MUFA**

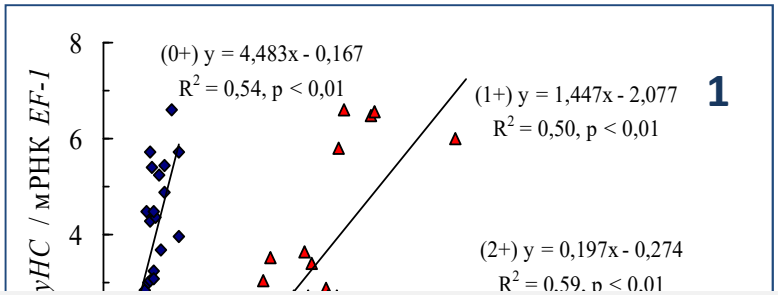
These data, together with a rise in **PS** (the most effective regulator of the activity of Na+,K+–ATPase – a key osmoregulatory enzyme) in **1+ parr** and, especially so, in **smolts** evidence the **formation of a biochemical mechanism of pre-adaptation to the marine environment**

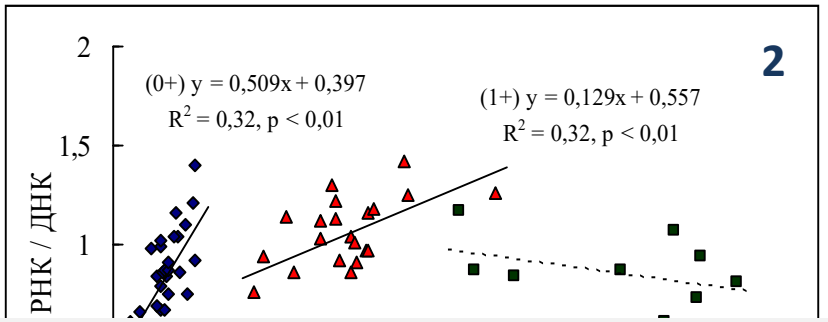
Certain fatty acids content (% total FA) in juveniles of Atlantic salmon (at 0+, 1+, 2+ ages) collected from Sobachji shoal (the Varzuga River)



Higher level of long-chain and essential **20:5ω-3 and 22:6ω-3**, and ratios **22:6ω-3/18:3ω-3 and ω-3/ω-6** in **1+ parr show the start of preparation on biochemical level to change in environment from freshwater to marine.**

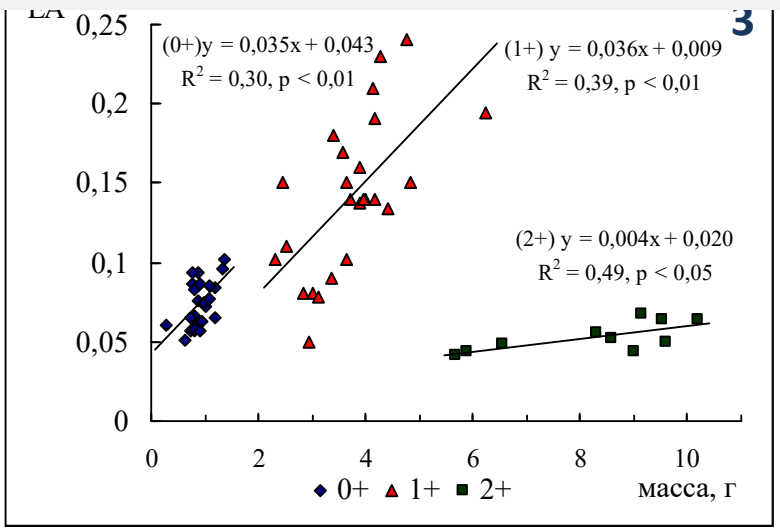
Relationship of the level of gene expression of *MyHC* (1), RNA/DNA (2), COX (3), LDH (4) in white muscles to individual mass of fish (*Salmo salar* L.) at 0+, 1+, 2+ ages

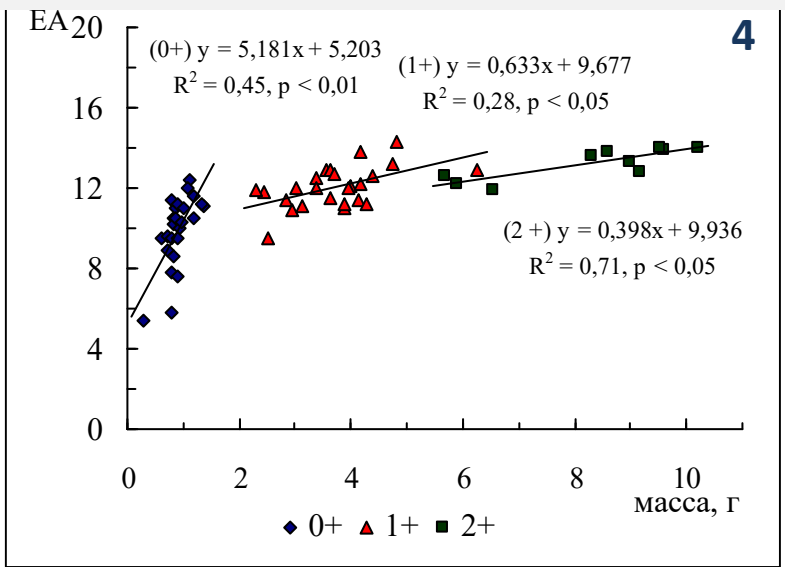




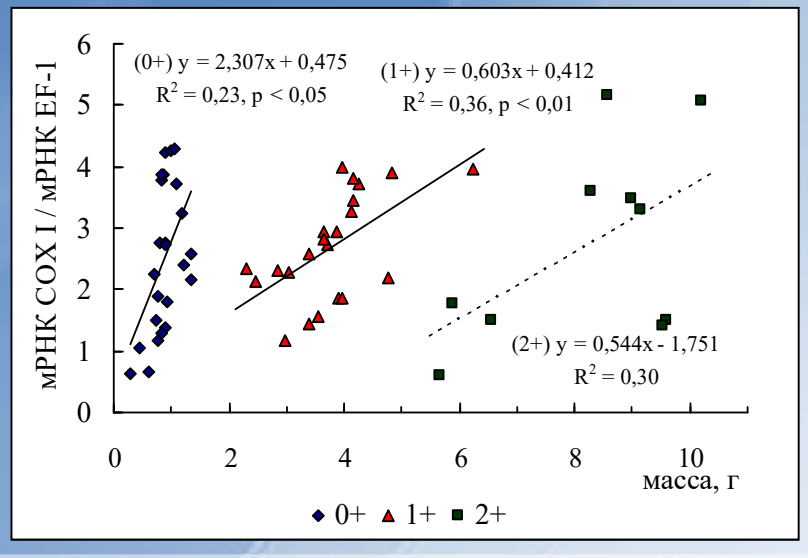
The highest level of myosin heavy chain gene expression and myogenic regulatory factors was demonstrated for **1+ parr**, pointing to a high rate of muscle tissue gain owing to both hyperplasia (formation of new fibers) and hypertrophy (enlargement of fibers)

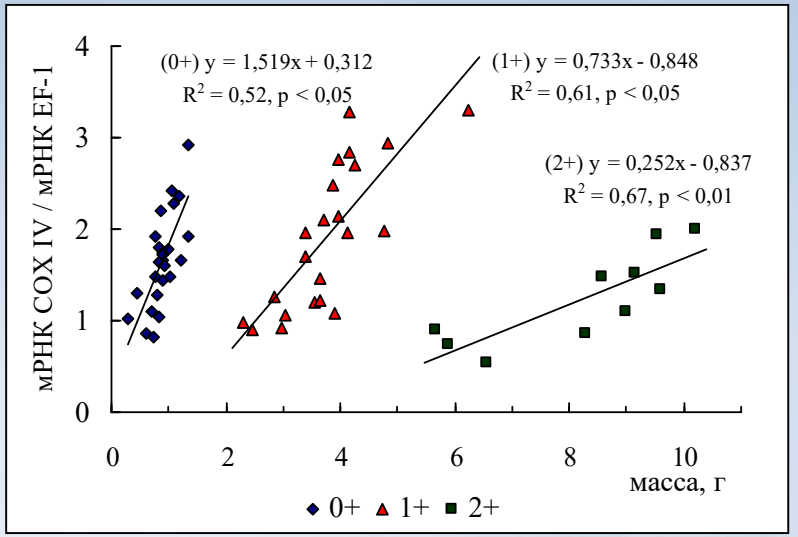
In older **parr**, the expression of these genes somewhat declined, suggesting a deceleration of growth rates with age



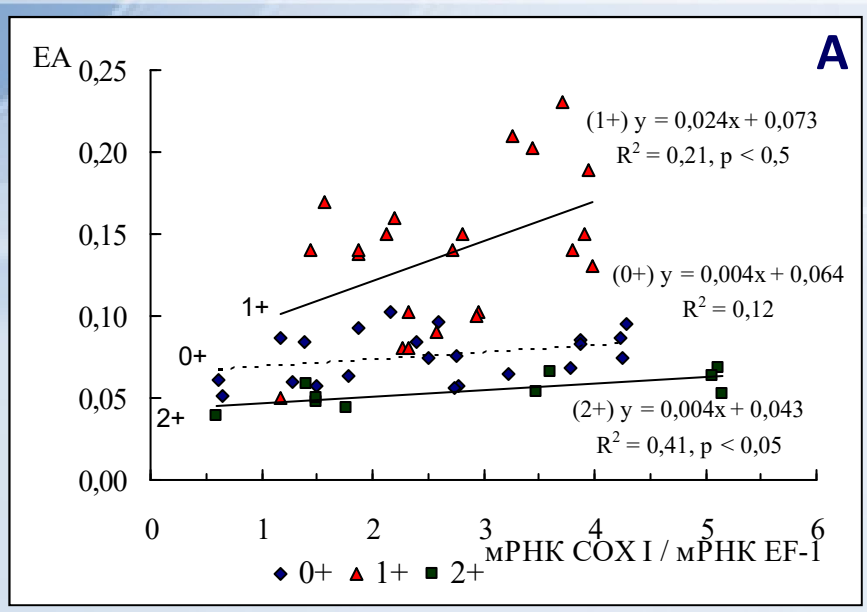


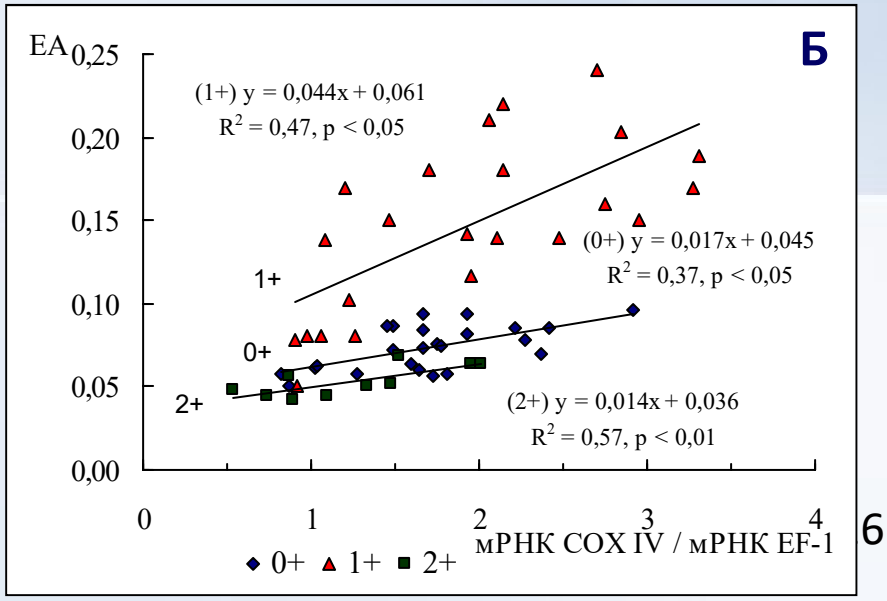
Relationship of the level of COX I (A) and COX IV(B) subunits genes expression to mass of juveniles at 0+, 1+, 2+



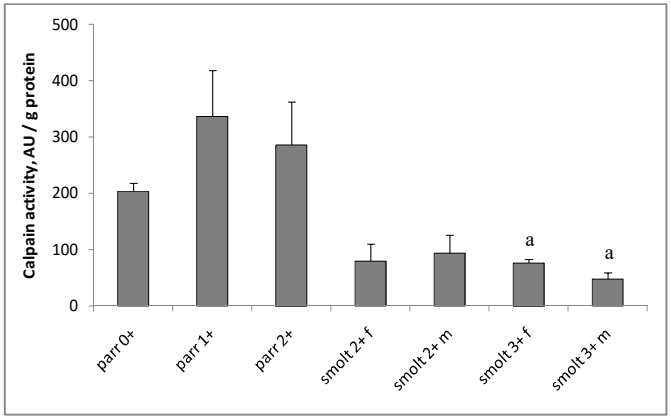


Relationship of the activity of COX and the level of COX I (A) and COX IV(B) subunits genes expression in the white muscles of juveniles at 0+, 1+, 2+ ages





ENZYMES OF INTRACELLULAR PROTEOLYSES OF JUVENILES *SALMO SALAR* L.

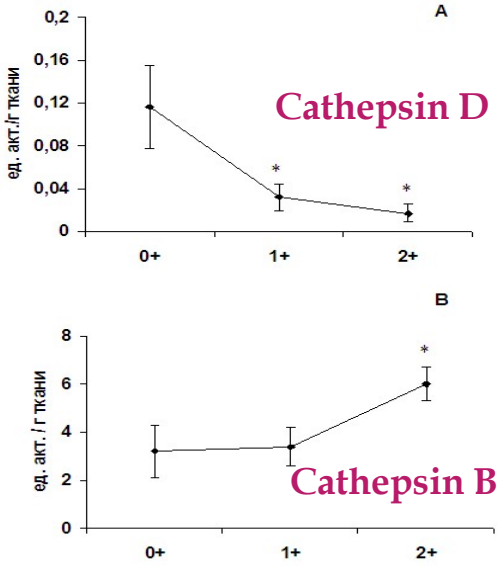


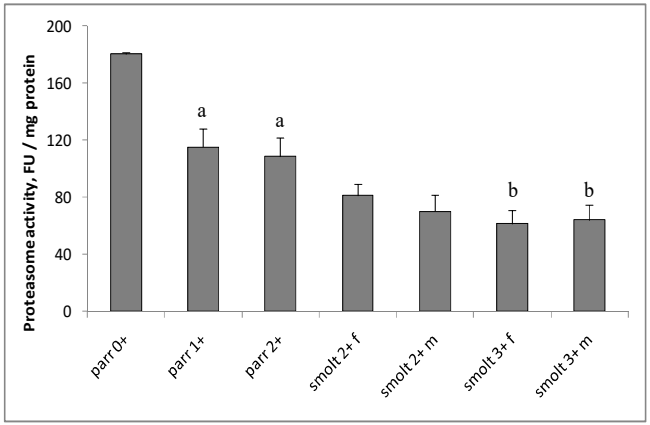
calpains

a –in distinction from parr 2+

The level of proteolytic activity in salmon parr positively correlates with the expression of *MyHC* genes and myogenic factors

Salmon smolts and parr of similar age vary reliably in terms of proteolytic activity but not myogenic potential





proteasome

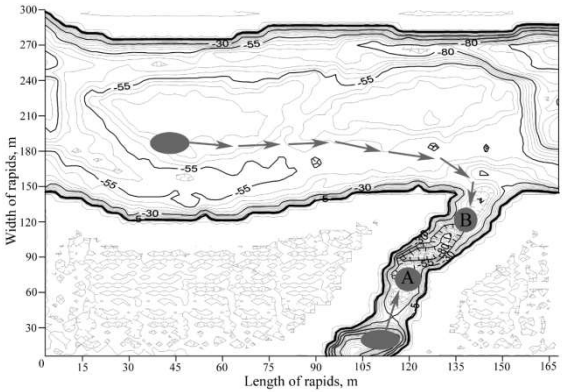
a – in distinction from parr 0+

b – in distinction from parr

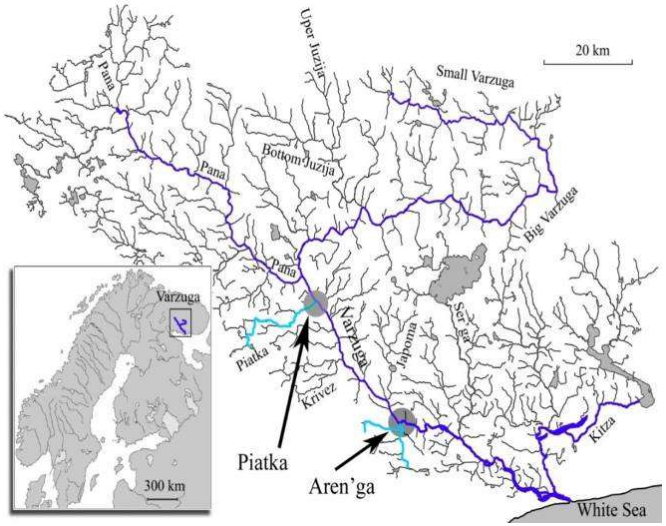
Age	Lenght, см	Weight, g
0+ fingerlings	5.64 ± 0.13	1.38 ± 0.09
1+ parr	9.75 ± 0.15	6.96 ± 0.31
2+ parr	11.56 ± 0.69	9.95 ± 0.86

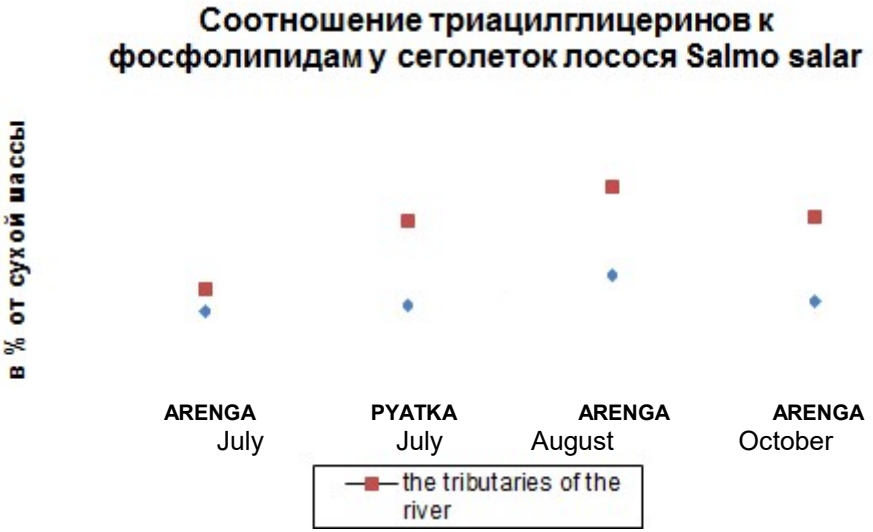
There is connection between the rate of growth and accumulation of the protein mass of juvenile salmon and the activity of intracellular proteolytic enzymes in their muscles

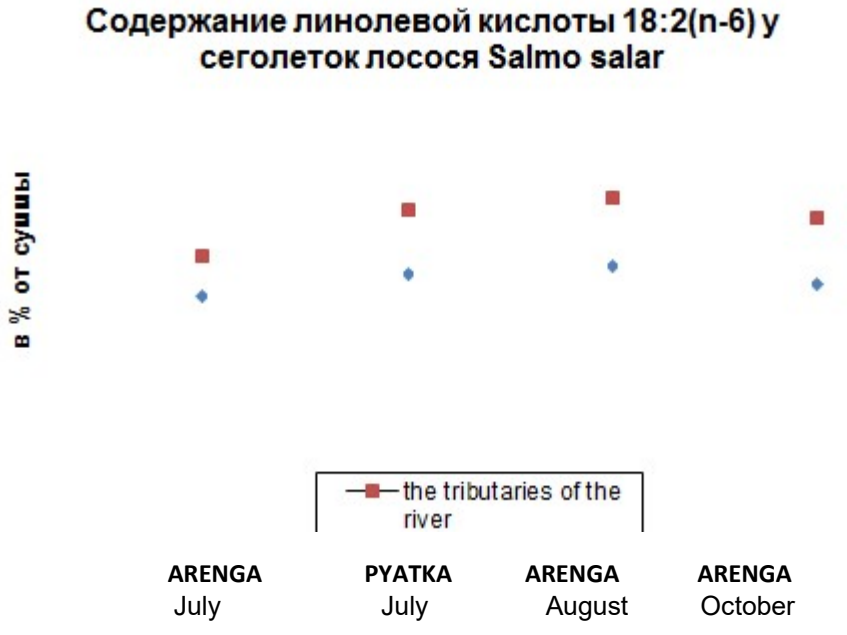
Bliochemical heterogeneity of fingerlings Atlantic salmon inhabited different biotopes of the *Varzuga River*

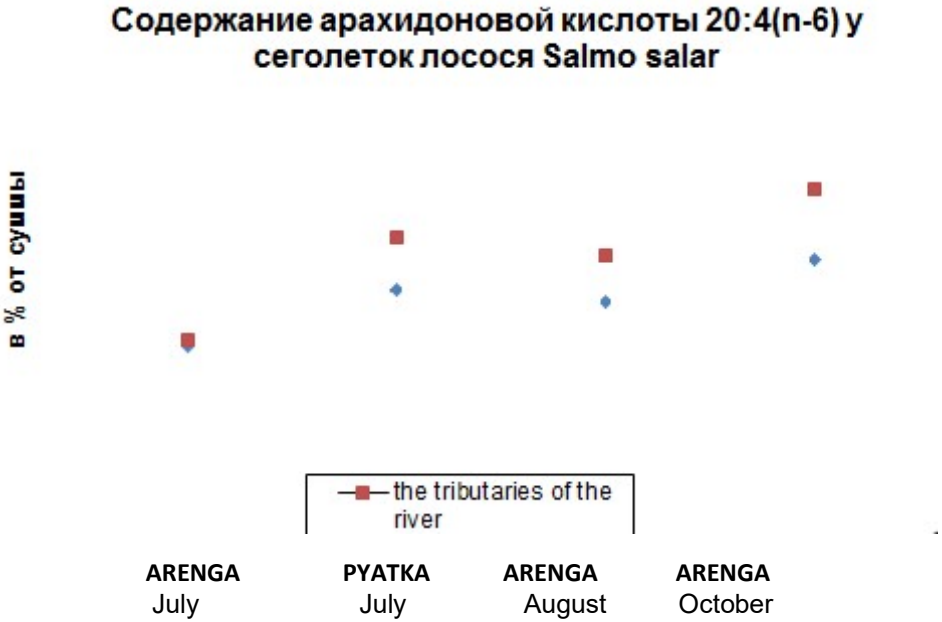


Lipids Heterogeneity of fingerlings Atlantic salmon from the biotopes of the mainstream of the Varzuga River and its tributaries









Lipids (% dry weight) and fatty acids content of fingerlings (0+) Salmo salar L. from different biotopes of the Varzuga River

Lipids, FA	tributaries of the river					
	Arenga		**Pyatka**		**Phalaley**	
TAG	1,09±0,15	2,02±0,10	**3,09±0,80**	3,29±0,70	0,12±1,20	3,99±0,80
CHOL	3,21±0,20	3,15±0,19*	3,36±0,30	3,87±0,40	5,67±1,00	3,05±0,70*
ECHOL	0,46±0,05	0,22±0,03*	0,74±0,10	0,73±0,20	0,51±0,10	0,53±0,08
CHOL/PL	0,70	0,60*	**0,76**	0,58*	1,11	1,56
TAG+ECHOL / PL+CHOL	0,28	0,27	**0,75**	0,38*	0,62	0,81
18:2ω -6	3,60±0,60	5,01±1,3*	5,62±0,8	7,00±0,40*	1,52±0,20	3,12±0,50*
20:4ω -6	0,20±0,10	0,21±0,10	1,73±0,5	2,11±0,70	1,32±0,20	3,01±0,40*
18:3ω -3	2,20±0,50	2,70±0,9*	**9,21±0,8**	7,92±0,60*	0,71±0,10	2,91±0,20*
22:6ω -3	13,30±1,9	11,90±1,6*	7,61±1,2	8,93±1,50	24,91±3,50	20,6±3,80
18:3ω-3/ 18:2ω-6	0,61	0,57	**1,58**	1,13*	0,46	0,94*

The most favourable conditions for fry growth and development were found in the **tributary Pyatka**
Fry there had the highest size and weight values. Fry from the tributary Pyatka had an elevated **TAG** level and higher T**AG+CE/PL+CHOL** ratios. Fry from the tributary Pyatka had an elevated **18:3ω-3/18:2ω-6 FA** level

These differences are primarily determined by the quality of the food reserve and the hydrological conditions of the accompared biotopes

.

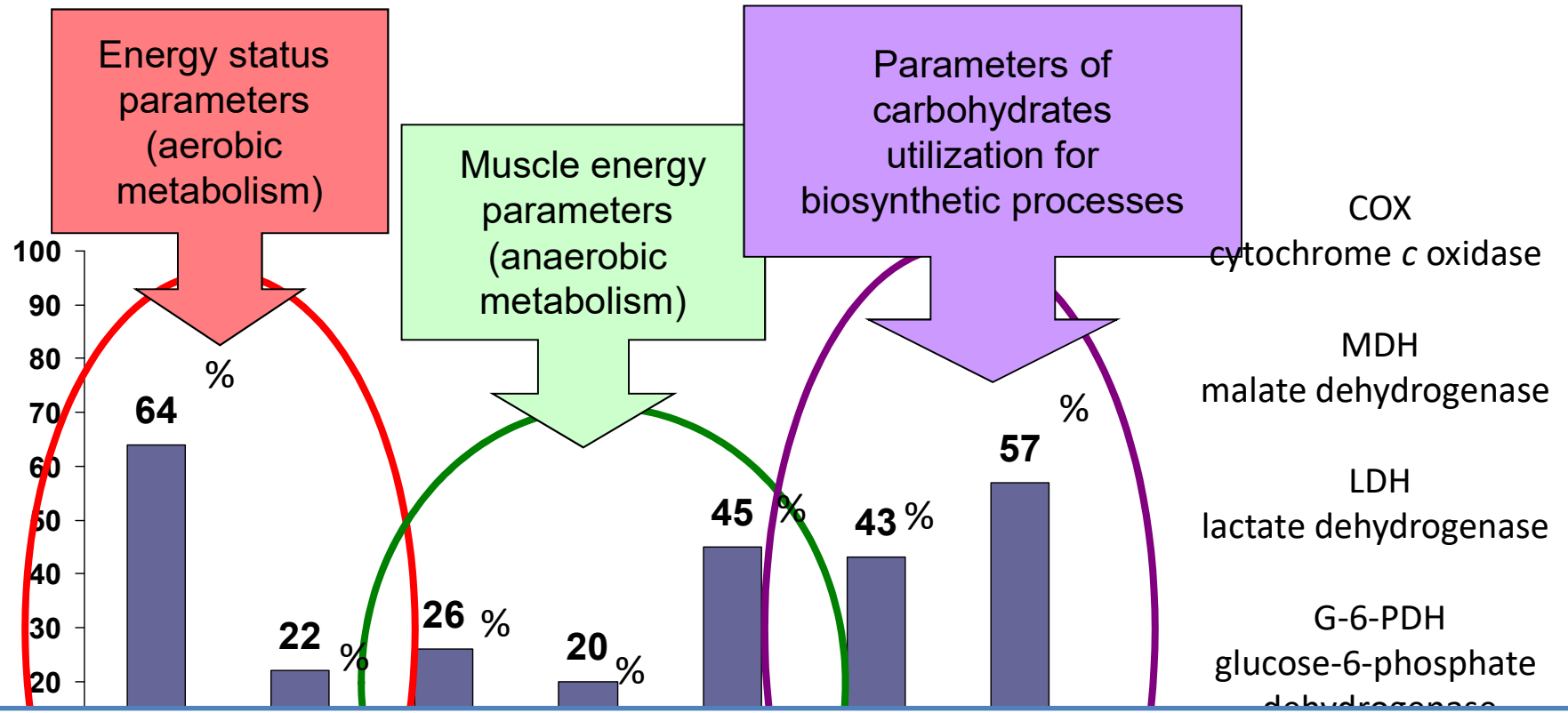
Activity of proteolytic enzymes (activity units) and protein content (mg/g tissue) in salmon fingerlings from the Varzuga mainstream and the Pyatka tributary

Fry living in the habitat with more favourable foraging and thermal conditions (tributary Pyatka), were found to have a higher activity of intracellular proteolytic enzymes, pointing to a higher rate of intracellular protein metabolism

	Biotope	
	Main stream Varzuga	**Tributary Pyatka**
Protein concentration, mg/g tissue	43,0±4,0	48,0±4,5
Total calpain activity (activity units)	0,53±0,05	**0,68**±0,06*
μ-calpain activity (activity units)	0,15±0,01	0,07±0,01*
m-calpain activity (activity units)	0,07±0,01	**0,40**±0,04*
Cathepsin D activity(activity units)	5,60±0,6	5,35±0,4
Cathepsin B activity (activity units)	42,10±4,0	**55,5**0±5,3*
Free hydroxyproline (μM/g tissue)	80,90±7,2	**118**,10±9,8*
Protein-bound hydroxyproline (μM/g tissue)	1260,50±107,5	**246**,20±20,7*

There is a correlation between changes in the activity of calpains and some parameters of the lipid status (phosphatidylinositol, arachidonic acid, fatty acid saturation/unsaturation ratio), which indirectly affect the activation of calcium-dependent proteolysis through modification of membrane microviscosity, and opening of ion channels for calcium transport to the cell

ACTIVITIES OF ENERGY AND CARBOHYDRATE METABIOLISM ENZYMES OF JUVENILES SALMON LIVING IN THE TRIBUTARY AREN'GA (N=30) PERCENT DEVIATION (ΔM, %) FROM PARAMETERS IN SALMON LIVING IN THE MAINSTREAM VARZUGA RIVER.



fry (0+) from the tributary feature:

increased energy production via both **aerobic** and **anaerobic** pathways;

increased synthesis of **glycerophosphate** (precursor of structural and storage lipids);

increased production of **pentoses** and **reduced NADP** for biosynthesis of nucleic acids and other compounds.

Aerobic ATP synthesis declines with age, while utilization of carbohydrates in anaerobic ATP synthesis increases and 1-GPDH activity rises

The biochemical parameters indicative of readiness for smoltification in brown trout and salmon were determined:

- *The role of **proteinases** in smoltification is species-specific.*

  *Inhibition of muscle protein breakdown serves to promote muscle build-up in **salmon** pre-smolts, whereas intensified muscle protein breakdown in **trout** pre-smolts saturates their tissues with osmolytes to help them survive in the marine environment until their osmoregulatory system has formed*

- *The principal ones are quantitative ratios of **CHOL/PL and TAG/PL, as well as EFA/PUFA, 16:0/18:1(n-9), 18:2n-6/18:3n-3, 22:6n-3/18:3n-3, 20:4n-6/18:2n-6***

- *Smolts of all age categories differ from parr in having a higher activity of **COX, LDH and 1-GPDH** in muscle and lower activity of enzymes of carbohydrate metabolism in liver*

CONCLUSION

The biochemical differentiation, which appears already at the earliest stages and persists in older age classes of juvenile fish, defines how the larvae and fry will **interact with** the environment, their resilience, survival, physical activity, migratory behavior and adaptation to the various ecological conditions

The distinctions detected in the biochemical status of salmon juveniles may be responsible for the extended duration (1-3 years) of the **parr-to-smolt transformation within the same generation** and, hence, for the earlier or later migration to the sea, thus being a factor for the formation of a complex age structure of the population

Pink salmon
Oncorhynchus gorbusha L.

The biochemical status was studies between:

1. **Groups of pink salmon the different parts of ovaries (jowl, central and tail)**

2. **Stage of development**

3. Two prolarvae developing in different nests under the same environmental conditions

Pink salmon *Oncorhynchus gorbusha* L. : different parts of ovaries

1. **In the forward parts of ovaries – higher amount of essential** 20:5(n-3) and 22:6(n-3) FA and ratio 16:0/18:1(n-9) that point on higher intensity of lipid metabolism; **in the central parts of ovaries** – lower level of TL due to PL (PC, PEA, SFM) and CHOL; **in the tail parts of ovaries** – higher level of PL due to PC, PEA, LysoPC, SFM, PI and CHOL.

No variability in parameters of carbohydrate metabolism and proteolysis in different parts of ovaries (individual) was found.

2. The results of the statistical analysis of the **whole eggs sample** collection demonstrate the roe **difference** in terms of **aldolase** activity (coefficient of variation - 67%), **LDH** (34%) and protein concentration (96%). Besides that, in relation to the whole eggs sample collection there is positive correlation between **LDH and aldolase** (r=0,50); **LDH and gonad mass** (r=0,56); **protein concentration and LDH** (r=0,49), **aldolase** (r=0,45), **gonad mass** (r=0,50).

No variability for proteolysis enzymes was found

Found heterogeneity in certain portions of ovaries indicated f asynchronous in biochemical processes in oocytes of these portions that in the end affects the ability to fertilization, growth and development of embryos and further differentiation of young fish.

Pink salmon *Oncorhynchus gorbusha* L. : different parts of ovaries

parameters	different parts of ovaries		
	jowl (*n*=4)	**central** (*n*=4)	**tail** (*n*=4)
weight, g	0.121 ± 0.006	0.127 ± 0.009	0.126 ± 0.007
Activity cathepsin B, (activity units)	6.9 ± 0.51	6.6 ± 0.80	6.9 ± 0.65
Activity cathepsin D (activity units)	0.9 ± 0.13	0.6 ± 0.15	0.9 ± 0.15
Activity calpains, (activity units)	87.44 ± 34.34	64.60 ± 35.60	45.81 ± 17.75
protein, mg/g tissue	2.1 ± 0.31	1.7 ± 0.18	2.0 ± 0.20
activity aldolase (activity units)	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Activity LDH (activity units)	0,23 ± 0.03	0.25 ± 0.02	0.27 ± 0.02

Pink salmon *Oncorhynchus gorbusha* L.

(different stages of its development)

	calpains	proteasomes	COX	LDH	1-GPDH	aldolase
in oocytes (V)	53.5±18.4	10.2±2.5	no	0,25±0,02	no	0,06±0,01
in embryos at the eye stage formation	93.1±15.0[a]	8.9±1.1	0,04±0,004	0,84±0,04	0,04±0,009	0,31±0,02
in larvae at hatching	127.4±24.3[a]	120.9±10.7[a]	1,30±0,03*	1,98±0,09	0,25±0,01*	43,92±3,84*

The results of the study indicate that the enzymes of intracellular proteolysis, energy and carbohydrate metabolism have a different degree of variability in their activity in the early ontogeny of pink salmon at different stages of its development

The biochemical differences between the two groups of pink salmon prolarvae developing in different nests under the same environmental conditions

(10 m from each other; the roe were put for development in different time periods, the difference made up one week; the average weight of 1 nest larvae - 1.3 g, and the weight of the 2-nd nest larvae - 1.6)

The differences were detected in the level of total lipids, including CHOL, CHOL/PL indicators, 18:3n-3/18:2n-6, 16:0/18:1n-9 FA, activity of enzymes of energy and carbohydrate metabolism (cytochrome c oxidase (COX), lactate dehydrogenase (LDH), aldolase and 1-glycero-phosphate dehydrogenase (1GPDG)

The reduced level of CHOL and, especially, CHOL/PL index in the prolarvae of the second nest indicates the decline of biomembrane viscosity and the increase of metabolic activity.

It should be particularly outlined that the prolarvae of two nests significantly differ by the content of total MUFAs, SFAs and PUFA of n-6 family, but do not differ by the content of total PUFAs, including PUFA of n-3 family

The metabolism of the larvae of the second nest has the higher level of aerobic metabolism, carbohydrate oxidation in the process of glycolysis and their transformation into glycerol phosphate (the precursor of the structural and reserve lipids)

These results are important for the prediction of the terms of hatching of pink salmon larvae from ovaries and leaving their spawning nests

When comparing the results of studies of lipid, carbohydrate and energy metabolism in relation to three species of salmonids
it was defined that brown trout had the lowest degree of difference,
and pink salmon - the highest

Pink salmon *Oncorhynchus gorbusha* L.

In the future, it will be interesting to see:

1. **Comparative studied of biochemical status of pink salmon and other species of Salmonids in the North to study biochemical adaptations and the role of some biochemical components in adaptation during embryogenesis and early ontogeny**

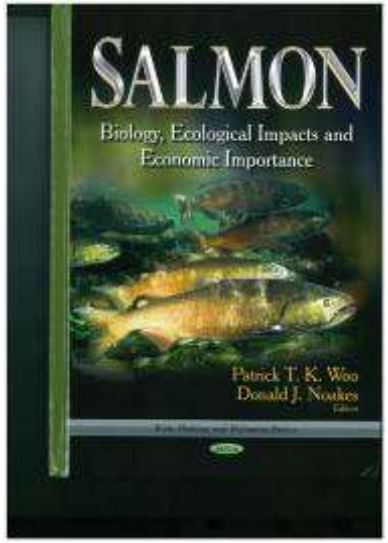
2. **Research the biochemical heterogeneity of pink salmon determines specifics in reproduction, growth and development, the strategy of life cycle, migrations, forms in population etc.**

3. **Study of the biochemical aspects of early development will allow a thorough understanding and assessment of the adaptive capacity of juvenile Pink salmon**

4. **To reveal ecological and biochemical aspects of relations and neighboring among Salmonids**

4. **Any else? Let's discuss**

Main publications in the frame of the presentation:

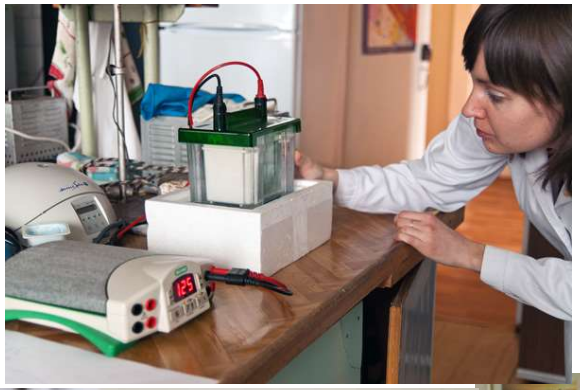


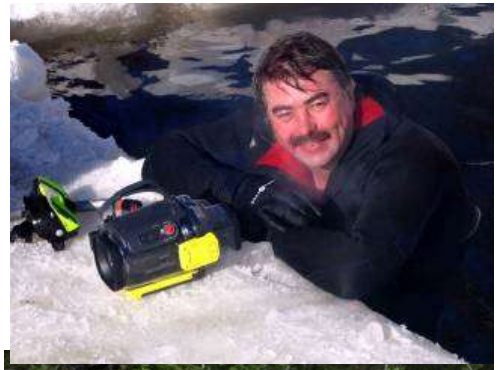
- **International Journal of Molecular Sciences** - 2013, 2015, 2016
- **Protein Science** – 2015, 2016, 2017
- **FEBS J. – 2016 (3), 2017 (3)**
- **Polar Record, 2016**
- **Comp. Physiol. Biocem., 2017 (2)**
- **Fish Physiol. Biocem., 2017 (2)**
- **Russian Journal of Ecology** 2015
- **Journal of Ichthyology (RAS)** 2007, 2008, 2009, 2010
- **Russian Journal of Developmental Biology** - 2009, 2010, 2012, 2015, 2016
- **Russian Journal Bioorg. Chem.** 2015, 2016
- **Biology Bulletin (RAS)** 2010, 2015, 2016, 2017
- **Doklady Biological Sciences (RAS)** 2012
- **Sibirskiy Ekologicheskiy Zhurnal-** 2016, 2017

http://biochemistry.krc.karelia.ru/section.php?plang=r&id=1282

co-authors of the studies whose results are reported in the presentation:

Murzina S.A.., Lyzenko L.A.., Churova M.V., Mecherjakova O.V.., Kancerova N.P., Nefedova Z.A., Pekkoeva S.N., Krupnova M.Ju., Ruokolainen T.R., Veselov A.E.., Efremov D.A., Ruchjov M.A., Bystrova K. A., Shulgina N.





http://biochemistry.krc.karelia.ru/section.php?plang=r&id=1282