Conclusions

The specific activity of squid proteases increases from young species to prespawning maturity stage species at pH 4.0, and then decreases significantly to the spawning stage species. At pH = 8.0, this activity is similar for young and pre-spawning species, but in the extracts of spawning squid also reduced in 2.5–3 times.

Upon squid maturing the pH-optimum of proteinases varies over a wide range – from 5.0 to 8.0 for the young species, in a narrow range – for the squids of spawning maturity stage, distinguishing by the gender: the pH-optimum of females – 7.5, males – 5.2.

By the method of zymogram it is found that chiefly enzymes with molecular mass of 55–60 kDa have gelatinous activity.

The study of the stability of squid proteinases at different pH values was carried out and a quite rapid loss of activity when increasing pH value to the alkaline region was shown.

PALE ARCTIC COMMON TAIMEN *HUCHO TAIMEN* WITHIN THE REPUBLIC OF SAKHA (YAKUT) – SPAWNING BIOLOGY AND ARTIFICIAL REPRODUCTION

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There are two species of taimen – common taimen *Hucho taimen* (Pallas, 1773) and Sakhalin one *H. perryi* (Brevoort, 1856), inhabiting rivers of the Russian Federation. Common taimen (or taimen) is one of the largest members of family Salmonidae that reaches 2.1 m in length, 105 kg in weight, and 60 years old in age. Its valid Latin name is *Hucho taimen* (Pallas, 1773) which has undergone five revisions made by P.S. Pallas himself. As a result this fish periodically changed its generic, specific or sub specific nomenclature. Therefore, the junior synonym is considered to be *Salmo fluviatilis*, the senior one – *Hucho hucho taimen,* other synonyms are *Salmo taimen* and *Salvelinus taimen* (Froese, Pauly, 2010).

In Russia, taimen is known under different local names: Russian – krasulya, len', talmen, Yakut – beal, Evenki – chonkchur (Sivtseva, Mikodina, 2009); in the English literature it is met as Siberian taimen and Siberian salmon. This species is an object of commercial fishing, game fishing and amateur one (Kirillov F.N., 1972; Kirillov A.F., 2002, 2009; Kirillov A.F. et al., 2007; Sidorov, Tyaptirgyanov, 2004) and aquaculture (Zelyonkin, Fedorova, 1997; Korablina, Ivanova, 2001; Mikodina, Lyubaev, 2005; Kouřil et al., 2009). Being a predator, taimen has delicacy meat and red caviar.

In the territory of the Republic of Sakha (Yakut) as part of Pale Arctic, taimen inhabits the rivers of the Arctic Ocean Basin running to the Laptev Sea. These are large rivers of different length – Undyulyung (*syn* Yundyulyun) R. (414 km), Omoloy R. (593 km), Yana R. (906 km), Anabar R. (939 km), Olenyok R. (2292 km), Lena R. (4400 km), and also south Lena River tributaries: left – Vilyui R. (2650 km), and right – Aldan R. (2273 km). Taimen habitats in Pale Arctic of the Yakut are extremely severe. For instance, the lower Lena R. with its tributaries is located not only beyond the North Polar Circle, but it is also found within the permafrost zone.

In Russia, taimen is included in the Red List Data of the Republic of Altay and Altay territory, Krasnoyarsk territory, Republic of Tyva, and Republic of Buryatiya. Its stock in the Yakut water systems steadily decreases, though it has not reached the critical level yet. In this connection, in this Republic, unlike other Russian Federation territories, it has not included in the Regional Red List Data yet. The stock reduction of taimen in the Yakut water systems is proved by the dynamics of catches (excepting the Great Patriotic War of 1941–1945): in 1940 – 25 tons, 1943 – 179 t, 1945 – 71 t, 1950 – 27 t, 1960 – 26 t, 1970 – 9 t, 1980 – 16 t, 1990 – 10 t, 2000 – 3 t. Since 1999, commercial fishing of taimen in the Republic is banned, and its fishery is allowed only as a by-fishing (10%) (see Sidorov, Tyaptirgyanov, 2004). According to the official statistics, in the zero years of XXI century its catches did not exceed 6 t. Thus, in 2006 the catches of taimen were 5 t, in 2007 – 3.9 t, in 2008 – 5.7 t, in 2009 – 5.98 t. By July, 2010 it has been caught 26.2 t, the Total Allowable Catch (TAC) for 2011 is estimated to be 28 t. Slightly less than half the TAC is allocated for recreational requirements, including the Lena R. – 8.5 t, in the Anabar and Olenyok Rs. together – 2.1 t.

The biology of taimen as an economically valuable species in the Yakut rivers is actively studied. It is a freshwater benthopelagic species. By the ecological classification it is considered to be a potamodromous fish, i.e. its migrations are carried out completely in fresh water (Froese, Pauly, 2010). It does not form great aggregations in the Lena R., being caught in this water basins only in summer (during the post spawning season) in the site between the settlement of Zhigansk and the settlement Jordjan, while in autumn – on the water sand grounds of Joldjongo and Searey-Kumakh (Sivtseva, Mikodina, 2008). During spring migration taimen comes for spawning into some Lena R. tributaries of the first and second orders. The most known sites of taimen spawning are found in the left (Motorchuna and Moona Rs.) and the right (Undyulyung R.) tributaries of the Lena R.

The limit age of the Yakut taimen, according to Kirillov (2002), is not less than 13 years old, average weight at this age is 8.1 kg; but individuals more than 80 kg in weight are met in Yakut. By our data, taimen females reach the age of 16+. Taimen becomes mature at the age of 7–8 years. In the wild it spawns in the late May-early June, its fecundity reaches 20 thousand eggs. More comprehensive and specified data on the reproductive biology taimen from the Anabar R. are shown in the monograph by Kirillov (2007): at the age of 7+ and 8+ years the taimen body length averages 617 and 687 mm, and its weight – 1850 and 2442 g, respectively.

We have studied the taimen biological and reproductive parameters from Motorchuna and Moona Rs. These Arctic Yakut rivers freeze in the end of September-October, and the ice in these rivers is broken up in the late May–first half of June. The taimen from the Motorchuna R. is larger, than that from the Moona R., but the age range of the fishes under study, according to our data, in the Moona R. was narrower (Table 1), that could be due to non-representative quantities of the individuals measured by us. In spite of this fact, in these rivers taimen is larger, than in other Siberian rivers (Reshetnikov, 2002).

Table 1. Age, Fork Length (AC) and Weight (W) of Common Taimen *Hucho taimen* from the Moona (n = 141) and Motorchuna (n = 24) Rs. during the Spawning Periods

Age, years	Moona R.						Motorchuna R.	
	2003		2004		2005		2008	
	AC, cm	W, kg	AC, cm	W, kg	AC, cm	W, kg	AC, cm	W, kg
5	71.0	3.0	–	–	–	–	–	–
6	74.5	3.0	89.7	7.1	–	–	94.0	6.0
7	83.0	5.2	96.7	7.8	94.2	8.3	93.3	8.6
8	105.0	9.8	103.6	9.5	100.0	9.3	103.1	9.3
9	102.0	9.6	110.5	10.9	109.7	11.2	111.8	16.3
10	106.0	9.8	117.5	13.3	113.0	13.0	–	–
11	108.6	10.7	–	–	119.7	14.5	115.0	22.0
12	113.8	11.5	–	–	–	–	–	–
13	113.0	11.9	–	–	–	–	–	–
14	115.0	14.0	–	–	127.0	17.8	–	–
15	116.0	14.0	–	–	–	–	–	–
16	121.0	17.0	–	–	–	–	–	–
17	124.0	24.0	–	–	–	–	–	–

The extention of taimen spawning migrations and location of it spawning grounds depend both of water regime of the spawning river, and of the Lower Lena R. basin as a whole. According to our data, taimen begins its upstream spawning migration to the Moona R. just after its cleaning from ice usually in May or in early June. For example, in 2003–2004 spawning taimen entered this river on the same days – June 4–5, when water temperature varied from 0.5 to 2.0°C, but in 2005 its run was very early (first half of May). As a rule, its mass spawning occurs in the second half of May. The taimen individuals caught during the spawning period have a nuptial dress: their abdomen and edges of fins are of intensive red color. Their sex ratio on the spawning grounds in different years varies, for example in 2003 in the Moona R. it was equal to 1:1, in 2004 – 0.6:1, in 2005 – 1:3. The condition coefficient (CC) (Table 2) of spawning fishes in the 2000ies fluctuated from 0.65 to 1.3%, that less, than in the middle of the 1980ies. On the spawning grounds the taimen groups are mixed and consist of immature and mature individuals. Thus, in the Moona R. during spawning season females and males with gonads of different maturity stages from II up to VI-II are caught, with predominance of prespawning migrants (51.1%) whose gonads are in the IV maturity stage, including 36.9% of females and 14.2% of males. There is a slight part of spontaneously spawned females (1.4%).

Table 2. Taimen Condition Coefficient (CC) during Spawning in the Moona R.,%

Parameters	Years			
	1986 **	2003	2004	2005
CC*	1.03 (0.84–1.18)	0.86 (0.65–1.3)	0.91 (0.8–1.1)	0.91 (0.75–1.05)
n	13	89	31	20

Notes. * – by Fulton, ** – Zhigansk Fish Inspection data.

Individual Absolute Fecundity (IAF) of taimen (Table 3) is rather high, on the average it is over 15 thousand eggs, the relative one is 1.7 eggs per kg. The fecundity increases with the age. In the first time spawning females the IAF varies from 7.5 up to 16 thousand eggs. In the repeated spawners in the age of 9 +, this reproductive coefficient increases. Yakut taimen produces large eggs (diameter is 5–9 mm) of dark-amber or orange color, which is laid in self-made redds located on the stone-pebbly and pebble-sandy but no oozed bottom at a depth about 0.5 m. According to the data by Venglinsky et alter (1987), its juveniles downstream migration from spawning grounds begins in the middle of June.

Table 3. Absolute Fecundity of Taimen in the Moona R., thousands of eggs

Age, years	Year					
	2003			2004		
	n	IAF		*n*	IAF	
		M	lim		M	lim
8 +	–	–	–	3	11.9	9.3–13.4
9 +	6	9.2	7.5–10.9	2	12.9	9.9–16.0
10 +	7	13.7	11.3–5.2	2	21.5	20.0–23.0
11 +	9	14.8	10.1–8.4	–	–	–
12 +	1	16.3	–	–	–	–
13 +	3	15.2	11.8–7.8	–	–	–
14 +	2	19.6	18.8–20.5	–	–	–
15 +	–	–	–	–	–	–
16 +	1	20.6	–	–	–	–
	29	15.6±1.4	7.5–20.5	7	15.4±3.1	9.3–23.0

Because of taimen catch reduction in Yakut in more than 70 times during 1943–2010, efforts are taken for increasing the stocks of this species by artificial reproduction. So the Government of the Republic of Sakha (Yakut) issued the decrée "On actions for artificial reproduction of aquatic living resources on the terrain of the Republic Sakha (Yakut) for 2007–2011", including taimen. This document was prepared by experts of the Yakut Ministry for Nature Protection and Yakut branch of Gosrybcenter, including one of present paper authors. Besides, the Regional Program "Reproduction of aquatic living resources in terrain of Yakut for 2011–2015" is being developed. According to this Program, release of common taimen juveniles from hatcheries to natural river basins by 2015 is to reach 200 thousand individuals (Sivtseva, Ivanova, Makarov, 2010).

To realize artificial reproduction of taimen there are all prerequisites in Yakut. Among them, biological and spawning data of this species, experience in artificial reproduction with the use of wild spawners are available, as well as the "Chernyshevsky" Hatchery at present being modernized. In 2009–2010 a legal procedure of this Hatchery transfer into the Federal property is carried out. Just at this hatchery, experimental studies on the elaboration of biotechnics of taimen artificial reproduction have been performed; they will be continued in future.

So far, foundations for artificial reproduction, on-growing and some aquaculture details have been created only for one of Russian taimen species – the Sakhalin one (Korablina, Ivanova, 2001; Mikodina, Lyubaev, 2005; Zelyonkin, Fedorova, 1997; Kouril et al., 2009). Nevertheless, taimen biotechnics artificial reproduction were based on two sources: instruction on artificial reproduction of Pacific salmons of genus *Oncorhynchus* (Smirnov, 1963, 1975), and Sakhalin taimen one.

During 2003–2005, taimen mature spawners were caught in the Moona R. wild spawning grounds, they were transported to temporary fish-breeding site and kept till ovulation. Here eggs and sperm were obtained for the first time without hormonal stimulation, 30 thousand eggs were inseminated, share of fertilized eggs reached 80%. Early stages of egg incubation was made on the net trays placed in the shallow quite river sites (Tyaptirgyanov, Ivanova, Sivtseva, 2008; Sivtseva, Mikodina, 2009). After that the developing taimen eggs from the Moona R. to the Hatchery "Chernyshevsky" (Mirninsky ulus), the unique in Yakut, were transported (in the beginning by air, then by motor and water transport). The Hatchery is specialized on cultivating cisco, peled, whitefish and their juveniles release to natural water bodies. The final incubation stages of developing taimen eggs were completed at the above Hatchery. Besides, the hatched prelarvae were ongrown in tanks during 30 days with the use of artificial feeds up to the weight of 500 mg. For the first time the taimen juveniles received by aquaculture methods were released to the Lena R.

The results of our Pale Arctic taimen studies on its reproductive biology and artificial reproduction under control conditions in the Republic of Sakha (Yakut) terrain, in our opinion, are an innovative trend in the Russian aquiculture. The accumulated experience makes it possible to rely on success in the maintenance and increase of common taimen population stocks using the methods of artificial reproduction not only in the Lena R., but also in other water systems.

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COMPARATIVE CHARACTERISTIC OF HYDROLYTIC ENZYMES OF THE BARENTS SEA INTRODUCED CRABS *CHIONOECETES OPILIO* AND *PARALITHODES CAMTSCHATICUS*

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Nowadays one of the key problems of the fish-processing industry in Russia is the development of new fishery objects and their rational use. Due to the increase of snow crab abundance in the Barents Sea, it is possible to predict the potential of this species for the industrial fishing. According to the data obtained by PINRO scientists, in 2009, the total stock of snow crab in the Barents Sea was more than 10 million individuals (Pavlov, 2010).

For several decades, hepatopancreas of the red king crab (*Paralithodes camtschaticus)* has been successfully used for production of complex enzymatic preparations applied in medicine and cosmetology, and also in food and microbiological branches of industry in order to obtain the protein hydrolyzates (Klimova et al., 1990; Mukhin and Novikov, 2001). The snow crab (*Chionoecetes opilio*) hepatopancreas, which has been insufficiently studied in this respect, is also of interest.

The objects of research were the enzymatic preparations derived from hepatopancreas of crustaceans *Paralithodes camtschaticus* and *Chionoecetes opilio*, caught in the different areas of the Barents Sea in 2008–2009.

To obtain enzymatic preparations (EP) the comminuted hepatopancreas was processed with acetone and n-butanol in order to remove lipids and low-molecular compounds (Sakharov et al., 1988).

The fractional composition of proteins in samples was determined by the method of low pressure gel-chromatography using «Pharmacia LKB Biotechnology» equipment. Sephadex G-100 Superfine was used as a stationary phase in a column (1,6x70 sm), 0.15 n NaCl (pH 7) – as an eluent buffer. The protein fractions were registered applying photometry at 280 nm (the optical path length – 2 mm). The molecular weight of proteins (MW) was determined using the calibration curves built after the run of the proteins with known MW through the column: thyroglobulin (670 kDa), g-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) as well as vitamin B12 (1,35 kDa) (Laurent and Killander, 1964).

Proteolytic activity was estimated using the Anson's method**,** by the hydrolysis of 1% sodium caseinate solution (Alekseenko, 1968). The temperature and pH values at which the protease activity was maximum were also determined.

Exochitinase activity was calculated through the release of N-acetylglucosamine (GlcNAc) generated by the chitin hydrolysis, the content of GlcNAc in a hydrolyzate solution was determined by the reaction with 4-dimethylaminobenzaldehyde (Decleire et al., 1996).

In the enzymatic preparations obtained from hepatopancreas of two crab species, *Paralithodes camtschaticus* and *Chionoecetes opilio,* chitinolythic and proteolytic activity has been determined. Seasonal dependence of protease activity of these two crustacean species has been found (Fig. 1). Thus, the activity level of EP in winter-spring period exceeded that one in summer-autumn for both crabs. The obtained results agree with the data of other researchers (Nemova, 1996; Mukhin and Novikov, 2002) and indicate the influence of seasonal rhythms on the activity of proteases. It may be caused by both the feeding pattern and the effect of the environment abiotic factors.

Considerable seasonal fluctuations of proteolytic activity in hepatopancreas of crabs indicate high adaptive abilities of the latter ones.

The molecular-weight composition of proteins in obtained EP for both crab species is quite similar (Fig. 2, Table). The high-molecular fraction makes up a considerable part of the total number of proteins in both samples.