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Spermal Activity and Change in Pacific Salmon Features

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Abstract. **The fishes progeny is the prospective to obtain in culture on the base of selected best gametes and best combinations of heredity factors, however this problem is complex. Also we considered important to find connection of gamete features with biological ones of progeny. The activity of sperms as feature, which can be influenced by selection, was studied. The methods of gamete selection (GS) which decreased rate of heredity defects were viewed.**

We explored progeny of chum salmon Oncorhynchus keta, pink salmon O. gorbuscha, chinook salmon O. tshawytscha and sockeye salmon O. nerka after gamete selection. Following features of progeny were described: survival; sexes ratio; fluctuating asymmetry (FA); diversity of features. FA is used as quality index of ontogenesis stability in development. We found that near 40-50% of chum salmon FA is controlled by heredity apparatus. The chum salmon (Sakhalin Island, Sokolovsky hatchery) and chinook salmon (Kamchatka, Malkinsky hatchery) researched in the presented work is characterized by high occurrence of fluctuating asymmetry.

Key words: gametes selection, sperms selection, sperms activity, salmons progeny, fluctuating asymmetry, survival, sexes ratio, diversity of features.

I. INTRODUCTION

Fish-culture of salmon increases abundance of survival progeny. However, its flaw is decrease of natural selection. The several forms of the natural selection are known and gamete selection is one of them. It was found [1] that sperms may be separated. Our investigations were prolonged aimed at study of spermal activity duration and factors influence on gamete selection. As selection factors we chose those which were close to natural factors. Spermal activity was studied at different conditions including physiological NaCl and KCl solutions forcing which were used by V.Ushakov and V.Vasilyeva [2] in the experiments with Salmo trutta. According to the authors [2] KCl is more severe factor that prevented fertilization of eggs by sperms with less life force.

II. MATERIALS AND METHODS

In presented report the material for studies of chum salmon Onchorhynchus keta, pink salmon O. gorbuscha was obtained at Sokolovskiy hatchery, in rivers Belaya, Bolshoy Takoy (Sakhalin Island), chinook salmon O. tshawytcha and sockeye salmon O. nerka - at Malkinskiy hatchery (Kamchatka), in river Bistraya, lake Dalnee in 1986-1996.

We studied spermal activity of chum, pink and chinook salmons.

There were studied the changes of spermal activity after storage of sperms during 4 days at temperature 0,5 ° C and progeny produced after fertilization of eggs by those sperms.

The application of gametes selection methods including dilution of gametes by freshwater are shortly described as follows:

1. Gametes selection-1 (GS1). Eggs were placed in vessel, then water was added at equal volume, mixed and after 5, 10 (12), 30, 60 sec sperms were added, mixed with eggs, kept for period of 3 min, washed out and placed for incubation.

2. Gamete selection procedure based on two steps addition (TSA) of sperms to water. The first part of sperms added into water, mixed, then added second part of sperms, all mixed with eggs, kept for period of 3 min, then usual procedure.

3. Gamete selection on the base of sperms dilution by physiological NaCl and KCl solutions. Sperms were diluted by physiological solutions NaCl or KCl and stayed for period of 5 or 6 min, then were mixed with eggs, then usual procedure.

The eggs fertilized applying dry methods are considered as control variant (control).

At insemination it is needed to take into account species peculiarities of salmons and quality of gametes (for example density of sperm). Fertilized eggs were incubated at hatchery**,** in the same place we reared fry. The O. nerka progeny was incubated at two regions: Malkinskiy and Paratunka hatchery.

Following features of progeny were described: survival of embryos, larvae and fry; ratio of males to females of fry; weight of fry; fluctuating asymmetry (FA); resistance to unfavorable environment. The row of coupled features was studied concerning FA: number of seismosensory channels pores, gill rakers number on fourth gill arc, rays in pectoral fins, spots along the side line.

III. ACTIVITY OF PACIFIC SALMON SPERMS

Sperms activity decreased at storage. After 24-26 hours storage we observed that ability to activation decreased to less than 0,5 % at pattern with liquid pink salmon sperm. After 3 days of storage the sperms activity was not registered in samples of liquid sperm.

Samples of dense sperm saved more ability to activation during two days. It was found that about 2% of sperms can be activated by freshwater after 26-28 hours storage. Some samples of dense sperm of pink salmon

saved ability to activation in following 4 days, however ratio of these sperms is very little, after 92 hours – less than 0,2%.

Before the description of data it needs to remind that insemination of salmon eggs takes place in water stream at natural conditions. Activity period of salmon sperms majority is very short in freshwater. Our investigations at this sphere will be described below. It is known [3] that eggs preserve fertility in freshwater longer than sperms.

According to our data sperms of O. keta lose motility in freshwater very quickly:

After dilution of sperms by river water at 8-10 ° C:
during 3-5 sec near 80% of sperms moved actively forward,
after 8-10 sec near 30-10% of sperms moved actively forward,
after 15 sec near 5-1% of sperms moved actively forward,
after 30 sec movement of vast number of sperms ceased,
after 55-sec progressive movement of almost all of sperms ceased.

The indices of pink salmon sperms activity from Sakhalin were similar. It is necessary to note that we described better pattern of salmon sperms.

We offer data concerning activity of chinook sperms in water of native river. After dilution of sperms by this water at 5-7° C:
during 15 sec 90-95% of chinook salmon sperms moved actively forward,
after 20-25 sec nearly 50-40% of sperms moved actively forward,
after 45-50 sec sperms moved forward seldom,
after 75 sec sperms moved forward very seldom.

Therefore activity of chinook salmon sperms was higher than that of chum and pink salmon. We suppose that this is connected with the fact that chinook salmons are spawning in districts of rivers having quicker flow.

It needs to note that the quality of sperms of individual males differed significantly. The duration of spermal motility and ratio of active sperms from dense salmon sperm are higher than from fluid one. Insemination of eggs using moist method increases ratio of dead eggs. Therefore straight transfer of natural spawning conditions in fish-culture would not be prospective.

At the Far East hatcheries the dry method of insemination is usually applied that provided high rate of fertilization of eggs, nearly 95%. (We used this method as control). We noted that in this case motion of sperms happened in environment with large quantity of ovarial liquid components that had some peculiarities.

Motility of sperms was observed in ovarial liquid diluted by half by freshwater at 8-10° C:
during 1 min all sperms (100%) of pink salmon moved actively forward**,**
after 1,5-2 min some sperms stopped movement,
after 3-6 min 30-50% of sperms moved but velocity of movement slowed down,
after 6 min most of sperms ended movement but some moved 10-16 min and longer.

Therefore duration of sperms movement was strong increased in diluted ovarial liquid, moreover, difference of spermal activity of various sperms during 1 min was absent (similar picture was observed for sperms of chum salmon). We supposed that at these conditions all sperms had equal chances for fertilization of eggs and it resulted in deterioration of quality indices of progeny.

It is known [5] that salmon sperms save fertility longer in physiological solutions. We studied activity of Pacific salmon sperms in marine water. Sperms of chum salmon and pink salmon do not move in marine water of 34 ‰. High activity of sperms was observed in diluted marine water of 5 ‰. The following picture was observed for chinook salmon sperms at 8-10 ° C:
during 3-4 min 100% of sperms moved quickly forward,
later loss of sperms activity was observed, however single ones moved in following 1 hour and seldom longer.

Chum salmon sperms were less active. We registered activity maximally in 80% sperms in 5 ‰ dilution of marine water.

We observed high activity of chinook salmon sperms in NaCl physiological solution at 5-7° C:
during 1-2 min nearly 90-95% of sperms moved forward quickly,
after 3-5 min nearly 80% of sperms moved forward and slowly,
after 7-15 min small number of sperms moved forward and slowly,
after 15-19 min single sperms moved forward and slowly.

We registered following activity chinook salmon sperms in KCl physiological solution at 5-7° C:
during 10-15 sec nearly 10-20% of sperms moved forward quickly,
after 30 sec movement ceased.

If chinook salmon sperms to dilute by physiological solution KCl and keep during 4-5 min and then add ovarial liquid we would observe movement of nearly 20% sperms in following 1,5 min. After 2,5 min some sperms moved forward, but slowly. The like picture was observed if sperms were diluted by KCl in following 6-10 min and then ovarial liquid was admixed.

IY. INFLUENCE OF SPERMAL SELECTION ON SALMON PROGENY

As it was described above the storage of sperms resulted to decrease of the ratio of sperms which can be activated in freshwater. The influence of sperms storage on progeny was examined with usage of dry method of insemination of eggs (table 1). The eggs were inseminated in control through 3 hours after sperms were sampled.

Increase of embryos mortality was not observed at eggs insemination by sperms which were saved during 34 hours but increase of females number was registered**.** Such increase we connect with predominant inactivation of Y-sperms at 34 hours period. Similar increase of females number was observed in variant №38 (storage of sperms during 36 hours) but also mortality of embryos was

increased. Fertility of sperms was also reduced after 92 hours storage (№37).

The effect of dilution of sperms by freshwater was studied by A.I. Smirnov [3]. The chum salmon sperm diluted by water already after 10 sec fertilized eggs by 6% less than sperms at dry method of fertilization. The pink salmon sperms diluted by water after 20 sec fertilized 60% and 70% of eggs in different experiments. The author did not study progeny that was produced after this operation. We investigated different variants of sperms dilution by water aimed at gamete selection. Under usage of GO1 method the freshwater was added to eggs and only over 5, 10 or more sec sperms were added. Number of dead embryos was little (even sometimes less than control) if eggs had been in water during 5-10 sec (table 2, 3). Apparently, gamete selection took place at this condition. If the water influence on eggs was magnified to 30 sec and more, the number of dead embryos would increased. It may be supposed that more active sperms which could fertilize eggs after exposure of eggs to freshwater during 5-10 sec created basis for appearance of more viable progeny.

The effect of GO1 method was more significant if number of dead embryos at control variants was more. It took place when for insemination there was used gametes of late spawning males and females. It should be remarked the following peculiarity of late spawning producers. According to experience of Kalininskiy hatchery fish-breeders the usage of the chum salmon gametes of such producers for fertilization and incubation leaded to increase of dead embryos number. Therefore their fertilized eggs usually were not incubated at Kalininskiy hatchery (personal report of director T.T. Kochetkov in 1979). (The breeders of Sokolovskiy hatchery worked similar). However, the culture of chum salmon without late spawning producers resulted to conversion of autumn chum salmon in summer ones at Kaliniskiy hatchery.

At the culture of chinook and coho salmon [5] also phenomenon of the spawning change to earlier periods was observed. It happened despite unfavorable temperature regime of freshwater reservoirs and hatcheries in these terms. The authors named this phenomenon inadvertent selection. Apparently the cause of such selection was the same, non-usage of producers which moved to spawning in the end of the period.

The best indices of fry survival were observed at usage of two steps sperms addition (TSA) for row of salmon fishes (table 3, 4, 6). The chum salmon females number was increased when applying TSA method in comparison with control ($p<0,001$), besides the rate of fluctuating asymmetry of pores and gill rakers decreased (table 5). We think that these features are biologically more important than FA of spots along side line which varied significantly, though the FA of spots was reduced also at application of TSA method.

Rate of fluctuating asymmetry is the criterion of ontogenesis stability in development. The chum and chinook salmon researched in the presented work is characterized by high occurrence of fluctuating asymmetry in control variants (table 5, 7).

Totally FA of biologically important features of chum salmon progeny was decreased to 60-50% with using of TSA method in comparison with that of progeny produced on the base of dry method of eggs inseminations. Therefore heredity controlled origin of near 40-50% of chum salmon FA**.**

The spermal selection influenced on variability of features. In the experiments with spermal selection the variability of spots number was reduced (Fig. 1). The same picture was observed in gill rakers and rays of pectoral fins (Fig. 2, 3). Consequently, spermal selection decreased salmon features diversity.

We observed that chum salmon fry produced on the base of the spermal selection (the application of TSA) were more stable to hypoxia and formaldehyde than control fry.

The usage of TSA method for chinook salmon led to similar effects (table 6,7). Moreover according to data of table 6 dry method of insemination of eggs was most negative for chinook salmon survival.

Gamete selection of chinook salmon was studied on base of different methods including influence of physiological NaCl and KCl solutions (table 6) which were applied for dilution of sperm [2]. The trend of the decrease of progeny mortality was observed after processing of sperms by NaCl solution. Similar exposure to KCl solution did not lead to the same effect. The best indices in relation to progeny mortality decrease were observed at usage of TSA method. In this conditions it was used natural influence of freshwater but limited time.

The data on mortality decrease were supplemented by data of analysis of fry fluctuation asymmetry (table 7). The rate of FA features was decreased in TSA variant. It was described above that the significant ratio of FA appeared as consequence of heredity defects. Also, usual increase of females number was observed**.** We noted that sexes ratio is important feature because increase of males number testifies degeneration of population [1].

The similar exposure of sockeye salmon gametes did not lead to repetition of described previously effects (table 8; table was reduced in view of big volum**e**). The method of TSA and selection on base of NaCl solution usage for sockeye salmon did not give advantages. The dilution of sperm by KCl solution during 5 min (№1) drove to death of significant number of progeny at early development stages. (However, increase of fry weight was observed.) In common, influence of KCl physiological solution was more negative for sockeye salmon sperms than for sperms of chinook salmon.

We explain described effects by less activity of sockeye salmon sperms in comparison with other researched salmons. These effects possibly connected with ecology peculiarities of sockeye salmon which are spawning in more quiet reservoirs where eggs may be fertilized by less active sperms. Presence of ovarial liquid components is more favorable for artificial fertilization of sockeye salmon.

TABLE I
INFLUENCE OF PINK SALMON SPERMS STORAGE AT TEMPERATURE 0,5 ° C ON SURVIVAL OF PROGENY AND FEMALES NUMBER (AFTER METHOD OF DRY INSEMENATION OF EGGS)

№№	Variants. Duration of storage of sperm, hour	Fertilized eggs, number	Mortality of eggs after fertilization, %	Mortality of embryos, larvae, %	Ratio of female, %
31	Control (3 h)	2038	1,7±0,2	4,8±0,5	42,4±2,9
36	34	1521	1,0±0,3	6,0±0,6	57,9±3,5
37	92	1707	25,3±1,1	52,0±1,2	44,8±3,3
38	36	573	8,7±1,4	28,3±1,9	56,2±4,2
39	32	881	1,4±0,4	5,7±0,6	46,0±4,3

TABLE 2
INFLUENCE OF CONDITION OF EGGS PROCESSING ON PROGENY SURVIVAL OF CHUM SALMON

№№	Variants. Processing of gametes before insemination	Fertilized eggs, number	Mortality of eggs after fertilization, %	Mortality of embryos, during incubation, %
1	Control	772	2,4±0,6	19,0±1,5
2	Stay of eggs in water during 5 sec	568	3,9±0,8	12,8±1,4
3	Control	2737	2,4±0,3	1,1±0,3
4	Stay of eggs in water during 10 sec	1405	0,6±0,2	2,2±0,5
5	Stay of eggs in water during 30 sec	1462	1,2±0,3	4,6±0,6
6	Stay of eggs in water during 60 sec	1520	1,5±0,3	20,0±1,5

TABLE 3
INFLUENCE OF CONDITION OF GAMETES PROCESSING ON SURVIVAL OF PINK SALMON PROGENY

№№	Variants. Processing of gametes before insemination	Fertilized eggs, number	Mortality of eggs after fertilization, %	Mortality of embryos, during incubation, %
11	Control	802	1,7±0,5	34,2±1,8
12	Stay of eggs in water during 10 sec	724	1,4+0,5	18,1+1,4
13	Stay of eggs in water during 30 sec	881	1,3+0,4	36,0+1,8
15	TSA	859	5,1+0,7	9,1+1,0

TABLE 4
INFLUENCE OF CONDITION OF GAMETES PROCESSING ON SURVIVAL AND FEMALES NUMBER OF PINK SALMON PROGENY

№№	Variants	Fertilized eggs, number	Mortality of eggs after fertilization, %	Mortality of embryos, larva during incubation, %	Number of females, %
5	Control	431	1,6±0,5	9,7±1,2	45,5±1,0
6	TSA	752	1,4±0,4	5,6±1,0	65,5±1,1

TABLE 5
INFLUENCE OF CONDITION OF GAMETES PROCESSING ON FA RATE AND FEMALE NUVBER OF CHUM SALMON PROGENY

№№	Variants. Processing of gametes before insemination	Studied fishes, number	Fluctuating asymmetry			Number of females, %
			Spots	Pores	Rakers	
27	Control	107	1,38	0,2	0,35	44,0±3,3
29	Stay of eggs in water during 12 sec	117	1,1	0,12	0,37	47,3±3,0
30	TSA	120	0,7	0,1	0,18	58,0±3,2
	Fry of river Belaya (wild)	66	1,22	O,21	0,35	52,2±4,8

TABLE 6
INFLUENCE OF CONDITION OF GAMETES PROCESSING ON SURVIVAL OF CHINOOK SALMON PROGENY

№№	Processing of gametes before insemination	Fertilized eggs, number	Mortality of embryos, larvae, fry, %
37	Control	940	59,0±1,6
31	TSA	2014	6,5±0,5
32	Stay of eggs in water during 10 sec	2318	20,0±0,8
36	Stay of sperms in NaCl solution during 6 min	983	56,3±1,6
33	Stay of sperms in KCl solution during 6 min	1127	99,9

TABLE 7
INFLUENCE OF CONDITIONS 0F GAMETES PROCESSING ON FA INDICES AND FEMALES NUMBER OF CHINOOK SALMON PROGENY

№ №	Processing of gametes before insemination	Studied fishes, number	FA of spots	FA of pore	FA of gill rakers	Number of females, %
37	Control	260	0,9	0,18	0,49	33,4±2
31	TSA	167	0,8	0,13	0,26	56,2±3
32	Stay of eggs in water during 10 sec	80	0,9	0,09	0,34	51,3±5
36	Stay of sperms in NaCl solution during 6 min	64	0,9	-	0,40	50,2±6

TABLE 8
INFLUENCE OF CONDITIONS 0F GAMETES PROCESSING ON SURVIVAL AND WEIGHT OF SOCKEYE SALMON PROGENY

№№	Processing of gametes before insemination	Fertilized eggs, number	Mortality of embryos, %	Mortality during the hatching, %	Mortality of larvae, fry, %	All number of dead specimens, %	Mean weight of fry, mg
Cultivation at Paratunka hatchery							
1	Stay of sperms in KCl solution during 5 min	1161	35,4	16,2	1,1	52,7	1569,0
2	TSA	1732	20,8	0,9	0,8	22,5	876,2
3	Control	1198	10,0	7,5	2,8	20,3	701,3
4	Stay of eggs in water during 10 sec	1731	7,7	6,9	0,9	15,5	760,0
Cultivation at Malkinskiy hatchery							
10	Stay of sperms in KCl solution during 6 min	924	50,0	13,5	19,2	82,7	630,9
11	Control	1856	6,0	17,2	3,6	26,8	601,6
12	Stay of sperms in NaCl solution during 6 min	1952	22,6	4,9	8,2	35,7	-
13	TSA	1853	8,5	3,9	1,9	14,3	594,8

Therefore, all studied four species of salmons are differed in relation to peculiarities of their spermal activity.

The eggs insemination of these salmons is likely to be different under hatchery conditions.

Y. CONCLUSION

Thus, effective gamete selection results to several phenomena: increase of progeny survival, viability, number of females, decrease of fluctuating asymmetry rate. We noted earlier [1] also increase of growth rate of chum salmon fry after gametes selection. We explained these phenomena supposing that significant number of heredity defects is displayed in sperms and it decreased their activity. Spermal selection based on the TSA method decreased a number of sperms with heredity defects capable to fertilize eggs, therefore the rate of heredity defects at progeny was lessened.

Apparently the gametes selection is effective relatively great and small heredity defects elimination. The last ones decrease viability and increase the rate of fluctuating asymmetry of fishes. In natural conditions fishes with small heredity defects may survive, however, slowly grow, develop and come at spawning later. Possibly late spawning salmons contained more defects in heredity apparatus than these of early spawning.

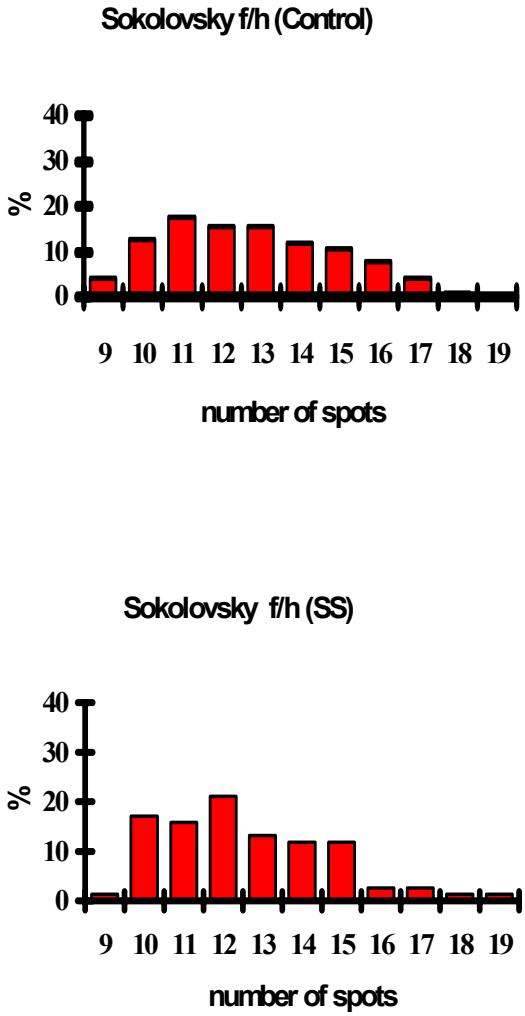

Fig.1.Variability of spots numbers of chum salmon

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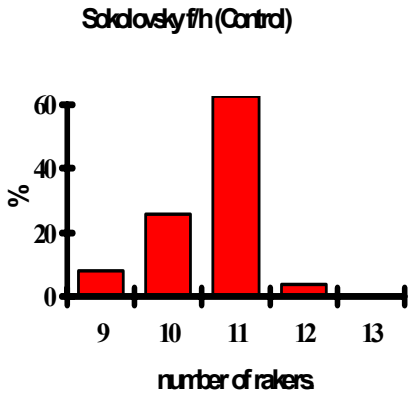

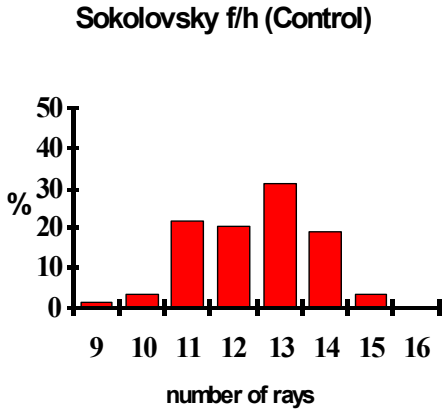

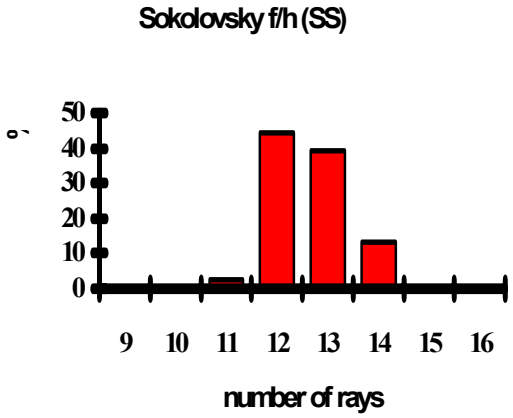

Fig. 2. Variability of gill rakers numbers of chum salmon

Fig. 3. Variability of rays numbers of chum salmon