ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. IV, Fasc. 2 Szczecin, 1974

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Embryology

SURVIVAL OF „DRY" SALMONID SPERM AT TEMPERATURES ABOVE 0° CENTIGRADE

PRZEŻYWALNOŚĆ „SUCHEJ" SPERMY RYB ŁOSOSIOWATYCH W TEMPERATURACH DODATNICH

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Survival of sperm from *Salmo trutta* L., *Salmo gairdneri* Rich., *Salvelinus fontinalis* (Mitch.) was investigated at constant temperatures (1.0°; 5.0°; 10.0°; 15.0°C). The longest period of survival has been found to take place at the temperature range from 1.0°–5.0°. Further it has been stated that the duration of sperm storage at various temperatures has only a slight influence on the activity time of spermatozoa. Differences have also been revealed with regard to various fish species, fish individuals, as well as individual spermatozoa.

INTRODUCTION

Several methods of storing fish sperm in its inactive state have been known thus far. They may be roughly subdivided into short term (from a few hours to several days) and long-term storage methods which usually call for freezing the sperm or keeping it at temperatures lower than 0°C in the presence of several protective media in order to have the possibility of using it in the consecutive reproductive season similarily as this is the case in zootechnics.

Experiments with storing sperm for a short time at various conditions of temperature, aeration etc. have been carried out on many fish species, mainly salmonid fishes, for some 100 years. All those works, however, have a considerable drawback. Now, most of them,

maybe with the exception of **Scheuring** (1924) are devoid of a comparative character in relation both to species under investigation, and to the research methods applied.

The efficacy of sperm is usually evaluated by two indices, namely the activity of the spermatozoa diluted with water (progressive movement) and their fertility, i.e., favourable results with fertilizing fish eggs – at best up to the eyeing stage) which obviously is a decisive test.

Although there is a certain correlation between those two indices, **Truscott** et al. (1968) drew attention that care had to be given not to take the activity of spermatozoa as a reliable indication of their fertility. Even earlier Žukinskij (1965) expressed the opinion that both those indices are to be treated as completely independent physiological phenomena. Thus the activity of spermatozoa is not to be considered as corresponding to their fertility. Since it is known that in some cases active spermatozoa do not ferfertilize eggs and those that have been found inactive under microscopic investigation fertilized them with excellent results (**Truscott** et al., 1968; **Ginsburg**, 1968; **Goryczko** and **Tomasik**, 1975). In spite of that, however, in practice the activity of spermatozoa is used as a quick test which, in conjunction with external appearance of the milt, permits to determine their suitability for further culture almost precisely.

In the foregoing work, (**Tomasik**, 1973) differences had been described in the activity of spermatozoa from several species of salmonid fishes during their storage at a constant temperature of 10°C. Therefore that was interesting to find out what happened at various temperatures of a value higher than 0°C.

Although the period of keeping up fertility during storage is dependent upon temperature according to **Ginsburg** (1968), such general statement, in spite of beeing obvious, is not sufficient. The necessity of carrying on research in this line seems to be fully justified in view of the recent studies connected with the transport of the reproductive products from salmonid fishes (**Cykowska** et. al., 1973) and their possible practical usefulness.

MATERIALS AND METHODS

Experiments were carried out on milt of *Salmo trutta* L. from the Rega river, *Salvelinus fontinalis* (Mitch.) from the hatchery IRS at Gdańsk-Oliwa and of *Salmo gairdneri* Rich. from the same hatchery as well as from the one at Mokre near Koszalin.

The subject of experiments was milt of a uniform consistency and of medium thickness taken from fourteen 3 to 4 years old males (4 *Salvelinus fontinalis,* 5 *Salmo trutta* and 5 *Salmo gairdneri*) under extremely clean conditions. Milt samples (separately from each male) were transported in dry glass jars, which had been placed in large thermos flasks. The temperature of the milt did not exceed 8° to 9°C during the transport. On arrival to the laboratory the milt from each individual male was divided into four parts and each part placed in identical dry glass ampoules and tightly sealed by means of corks. The ratio of the milt volume to the capacity of the ampoule was 1:4. The particular ampoules were stored at constant temperatures (1.0°; 5.0°; 10.0°; 15.0°C).

At 12 hour intervals, samples of milt were taken out and the activity of spermatozoa was tested in tap water at about 16°C. Both the total duration of their activity and the duration of the particular phases of activity (progressive and oscillating movement) were recorded as well as of those cases when only a small number of the spermatozoa or single ones were active. An aproximate correlation between the active and inactive spermatozoa (all or nearly all active; nearly a half of the spermatozoa active; only some of them active; only individuals active) was also determined.

The activity investigation of spermatozoa was carried out in accordance with such method as described in our previous paper (**Tomasik**, 1973). A sample of approximately 1 ml was taken from the central part of the milt surface and then replaced on a glas by a wooden stick with a blunt end. The moments at which the movement began and ceased and its particular phases were determined my means of a stop-watch. The measurements were repeated three times and average values calculated.

RESULTS

The obtained results indicate with no doubt that the temperature at which milt is stored after spawning has an essential influence upon its survival rate. The survival rate of spermatozoa after storage seems to be inversely proportional to the temperature value, at least within the temperature range of the experiments (at lower temperature of a longer duration, and at higher temperatures the viability is shorter). This may be seen distinctly in Table 1 in which the average results have been compiled of experiments on sperm from representative individuals of the given fish species.

The milt from individuals of various species has been found to behave in different ways. Namely, differences have been observed regarding various species as far as the duration of survival rate is concerned at various temperatures.

In addition to that, the experiments revealed quite considerable individual differences as regards to the influence of temperature on milt within the particular species (in spite of using nearly the same milt parameters as far as it was possible). Those differences referred to the duration of spermatozoa survival rate, as well as to maintaining relative spermatozoa vitality in the milt.

In addition to the differences connected with various species and individuals, there are also differences in the behaviour of individual spermatozoa. Namely some spermatozoa, after having been subjected to a prolonged influence of temperature, behave in a distinctly different way than the others. For instance, they begin to move after a few seconds when introduced into a drop of water or perform oscillatory movements lasting up to several minutes in some cases.

Finally the experiments proved that within the studied range of sperm storage temperatures (1.0°–15.0°C) the total duration of the spermatozoa movement, including that at the various phases, measured at identical temperatures shows only negligible deviations.

Changes in the activity of spermatoza in the sperm storage period at 1, 5, 10 and 15°C
(progressive movement in the numerator and oscillatory movement in the denominator)

Table 1

Species	Temperature	Time after spawning in hours															
		0	12	24	36	48	60	72	84	96	108	120	132	144	156	168	180	192
Salmo trutta	1	$61\frac{41}{20}$	$59\frac{36}{23}$	$50\frac{33}{17}$	$48\frac{32}{16}$	$45\frac{28}{17}$	$44\frac{24}{20}$	$40\frac{22}{18}$	$41\frac{23^*}{18}$	$39\frac{21^*}{18}$	$40\frac{21^*}{19}$	$38\frac{19^*}{19}$	=	=	=	=	=	
	5		$63\frac{42}{21}$	$46\frac{30}{16}$	$48\frac{34}{14}$	$38\frac{27}{11}$	$41\frac{30}{11}$	=	=	=	=	=	=					
	10		~~$58\frac{40}{18}$~~	$58\frac{37}{21}$	$46\frac{31}{15}$	$41\frac{28}{13}$	$38\frac{26^*}{12}$	=	=	=								
	15		$55\frac{39}{16}$	$48\frac{30}{18}$	$46\frac{26^*}{20}$	=												
Salmo gairdneri	1	$51\frac{28}{23}$	$45\frac{28}{17}$	$48\frac{25}{23}$	$44\frac{25}{19}$	$45\frac{25}{20}$	$46\frac{24}{22}$	$44\frac{26}{18}$	$43\frac{23^*}{20}$	$44\frac{22^*}{22}$	$38\frac{20^*}{18}$	$35\frac{21^*}{14}$	$34\frac{20^*}{14}$	$35\frac{18^*}{17}$	=			
	5		$48\frac{26}{22}$	$50\frac{27}{23}$	$47\frac{28}{19}$	$46\frac{26^*}{20}$	$44\frac{25^*}{19}$	$45\frac{23^*}{22}$	$43\frac{23^*}{20}$	$44\frac{21^*}{23}$	$40\frac{23^*}{17}$	$37\frac{24^*}{13}$						
	10		$47\frac{26}{21}$	$55\frac{21}{34}$	$51\frac{20}{31}$	=	=	=	=									
	15		$45\frac{25}{20}$	$42\frac{26}{16}$	~~$44\frac{25^*}{19}$~~	=	=											
Salvelinus fontinalis	1	$72\frac{33}{39}$	$70\frac{35}{35}$	$52\frac{31}{21}$	$46\frac{25}{21}$	$45\frac{27}{18}$	$39\frac{26}{13}$	$39\frac{27^*}{12}$	$42\frac{24^*}{18}$	$41\frac{25^*}{16}$	$42\frac{24^*}{18}$	$40\frac{22^*}{18}$	=					
	5		$64\frac{34}{30}$	$50\frac{25}{25}$	$46\frac{27^*}{19}$	$44\frac{29^*}{15}$	$35\frac{20^*}{15}$	$38\frac{25^*}{13}$	$44\frac{20^*}{24}$	–	–							
	10		$61\frac{28}{33}$	$60\frac{27}{33}$	=	=	=	=										
	15		$58\frac{34}{24}$	$51\frac{31^*}{20}$	$64\frac{32^*}{32}$	–												

* – about half the spermatozoa move
= – only some spermatozoa move
– – single spermatozoa move

DISCUSSION AND CONCLUSIONS

The results obtained from the present work permit to conclude unequivocally that the temperature at which the sperm is stored has an essential influence on the duration of keeping up the vitality of spermatozoa. Thus they justify and supplement the previous investigation into the possibility of fertilizing salmonid fish eggs at a later date than the natural spawning run (**Scheuring**, 1923, 1924; **Brofeldt**, 1923; **Poon** and **Johnson**, 1970; **Cykowska** et al., 1973).

The lower the storage temperature, the longer is the vitality of spermatozoa. Thus determined conclusions regarding the methods and ways of transporting the milt may be applied by fish culturists.

The method applied for the investigation into the activity of spermatozoa at the particular stages under uniform temperature conditions regardless of the milt storing temperature permits to draw clear conclusions on the influence of the latter on the activity of spermatozoa. In this respect there are only partial experiments available, namely those by **Scheuring** (1924) on milt of *Salmo gairdneri* Rich. as well as on milt of *Salvelinus fontinalis* (Mitch.) and *Hucho hucho* (**Scheuring**, 1928) and by **Lindroth** (1946) on milt of pike. Those authors, however, have not given any information on the water temperature, at which the experiments were carried out, and in the case of salmonid fishes the experiments were restricted to one storage temperature only. In addition to that, *Lindroth's* observations reffered merely to the total activity period without any subdivision into phases. Further, those authors conclude that the activity of spermatozoa under investigation undergoes no essential changes in the sperm during nearly the whole storage period at a given temperature and that it is only the final stage that it diminishes quickly. Conclusions of that kind have not been confirmed neither by our present experiments nor by our previous ones (**Tomasik**, 1973) regarding spermatozoa of salmonid fishes stored at 10.0°C.

A general phenomenon observed is that of a diminishing number of activating spermatozoa as well as of the period of their activity with the proceeding duration of storage after about two days time with differences appearing in the various species and individual fishes. Phenomena such as described by the above mentioned authors have been observed only in exceptional cases.

As far as the experiments of this work are concerned it is of interest to note that the duration of storing spermatozoa at various temperatures has only a minimum influence upon the time of their activity after coming into contact with water. The differences observed refer rather to shifting of the particular activity phase lengths. The differences as observed during the experiments regarding the particular species on the background of considerable differences connected with individuals may be in fact caused by a different stage of maturity of gonads. This may be explained in the course of further research work.

Interesting phenomena calling for further studies are also those of the considerable differences between individual spermatozoa, appearing as their prolonged inactivity phase

when coming into contact with water, as well as the differences between the lengths of their oscillating movements, and an earlier loss of their activity compared with other individuals.

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PRZEŻYWALNOŚĆ „SUCHEJ" SPERMY RYB ŁOSOSIOWATYCH W TEMPERATURACH DODATNICH

Streszczenie

Badano przeżywalność spermy *Salmo trutta* L., *Salmo gairdneri* Rich., *Salvelinus fontinalis* (Mitch.) w stałych temperaturach (1,0°; 5,0°; 10,0°; 15,0°C). Stwierdzono, że najdłużej utrzymuje się ona w przedziale temperatur 1,0°–5,0°C. Ustalono, że długość przechowywania spermy w różnych temperaturach w nieznacznym tylko stopniu wpływa na czas ruchliwości plemników, a także ujawniono różnice gatunkowe, osobnicze, jak i indywidualne.

ВЫЖИВАЕМОСТЬ „СУХОЙ" СПЕРМЫ ЛОСОСЕВЫХ РЫБ В ТЕМПЕРАТУРАХ ВЫШЕ НУЛЯ

Р е з ю м е

Изучали переживаемость молок **Salmo trutta L., Salmo gairdneri Rich., Salvelinus fontinalis (Mitch.)** в постоянных температурах (I,0°; 5,0°; I0,0°; I5,0°C). Нашли, что найдольше сохраняется она в пределах температуры I,0--5,0°C. Установили, что длина хранения молок в различных температурах в незначительной лишь степени влияет на время подвижности спермиев, а также показали видовые, индывидуальные и единичные различия.

Received: 18 VII 1974 r.

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